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Clinical Predictivity of Transplantable Tumor Systems in the Selection of New Drugs for Solid Tumors: Rationale for a Three-Stage Strategy[1,2]

Maurice J. Staquet,[3] David P. Byar,[4] Sylvan B. Green,[4,*] and M. Rozencweig[5,6]

The purpose of this work is to analyze the strategy used for the discovery of potentially clinically effective drugs by means of transplantable tumor systems in mice. With the present strategy used by the National Cancer Institute and the European Organization for Research on Treatment of Cancer, most new compounds are initially tested in the P388 leukemia model. Drugs showing activity in this prescreen are subsequently evaluated in a panel of eight screens (B16, CD8, Co38, L1210, LL, CX-1, LX-1, and MX-1). Positivity in any one of these screens qualifies a new drug for further preclinical studies leading to phase I–II clinical trial. Our analyses of the experimental screening data for 1949 compounds, and the comparison of results in humans and mice for 69 drugs that have been clinically evaluated against solid tumors, have indicated that the correlation of screening and clinical results is low and that a modified approach using four systems (P388, B16, L1210, and MX-1) would have been sufficient to uncover most of the drugs presently shown to be clinically active. In order to take advantage also of the information provided by other screens of the panel, a three-stage strategy can be used. In the first stage, compounds would be tested in the P388 prescreen, with a slightly higher level of response being required (T/C% $\geq$ 125 instead of 120). In the second stage, prescreen-positive drugs would be tested in the B16, L1210, and MX-1 systems. Drugs positive in any one of these screens may then be tested in Co38, LL, and CX-1 to determine priorities for clinical study. For new chemical structures of particular promise, this third stage could be dropped. LX-1 and CD8 are not useful in this context. Such a strategy would be much more economical and faster than the automatic use of nine systems, and the resultant savings should permit the discovery of an increased number of potentially active compounds. [Cancer Treat Rep 67:753–765, 1983]

Regardless of their origin, most anticancer drugs evaluated clinically have been investigated on the basis of experimental results for transplanted tumors in mice. Since 1955, > 600,000 natural and synthetic products have been tested in a variety of tumor systems to find new therapies for the control of human cancer. This undertaking has relied on the unproven assumption that there is a positive correlation between activity against transplanted tumors in mice and therapeutic effectiveness in human cancer. If this correlation exists, then the use of experimental systems for selecting drugs to be evaluated clinically is better than a random selection. Although many theoretic, pharmacologic, and statistical arguments can be advanced in favor of such a correlation, the yield of clinically effective drugs, let alone of curative agents, has been extremely low so far, reflecting either a poor predictivity of selection strategies or a low number of active compounds that actually exist.

The present screening strategy (1) introduced by the National Cancer Institute (NCI) in 1975 was designed to permit an evaluation of a number of screens forming the NCI tumor panel, after elimination of a large number of supposedly clinically inactive drugs by the P388 leukemia model used as a prescreen.

[1]Received Aug 20, 1982; revised Feb 10, 1983; accepted Mar 17, 1983.

[2]Animal tumor data used in the present analysis were generated in screening laboratories under contract to the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]European Organization for Research on Treatment of Cancer, Data Center, Brussels, Belgium.

[4]Biometry Branch, National Cancer Institute, Bethesda, MD.

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[6]We are grateful to the Developmental Therapeutics Program staff for their cooperation in providing computerized summary screening data to us, and J. Paolella and D. Eeckhoudt for typing the manuscript.

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It is recognized that a perfect evaluation of the predictivity of such screening systems cannot be carried out, because clinical data are lacking for drugs that are negative in all animal models. Also, the historical predominance of L1210 leukemia (2), considered for a long time to be the most dependable predictive model, has eliminated many drugs that are negative to L1210 without further testing in other systems (table 1). One result of this strategy has been that more clinically evaluated drugs, and therefore more clinically active drugs, are positive in the L1210 model than in other models, leading some workers to conclude incorrectly that this screen is superior to the others.

The purpose of this paper is to analyze the experimental data collected by the NCI since the beginning of the present screening strategy, and to examine its clinical predictivity for certain solid tumors. The assumptions, limitations, and biases of this type of evaluation will be explored. Finally, the practical meaning of the findings will be discussed, and a more efficient multistage strategy that identifies almost all of the drugs found by the present strategy will be proposed.

THE PRESENT SCREENING STRATEGY

With the present screening strategy (1), most new synthetic agents and natural products are initially tested in the P388 leukemia prescreen. Agents showing a percent increase in lifespan $\geq$ 20% over control on two occasions are tested in the entire panel of eight transplanted tumors (appendix I). A discussion of the NCI tumor panel, along with information on drug route and schedule, has been given by Venditti (3). A number of prescreen-positive drugs, such as analogs, are not sent to the panel in spite of their P388 results. Also, a number of agents (approximately 10%) referred to as "bypasses" are tested in the panel regardless of their P388 results. These compounds are of interest because of reported activity in other screening programs, or because of special chemical or biologic features. Some have already been clinically evaluated elsewhere.

TABLE 1.—Historical evolution of in vivo screens at the NCI*

Year	In vivo screen
1955	Sarcoma 180; carcinoma 755; leukemia L1210
1960	L1210 + 2 models from a pool of 21
1965	L1210 + Walker carcinosarcoma 256
1968	L1210 for synthetics; L1210 and leukemia P388 for natural products
1972	L1210 for synthetics; P388 for natural products; B16 melanoma and Lewis lung carcinoma for special testing
1975	P388 as prescreen, followed by a panel of 8 models, including human xenografts

*J. M. Venditti, personal communication.

Positivity in any one of the eight screens of the panel qualifies a drug for further preclinical investigations leading to phase I–II clinical trials. The selection process is monitored at various stages by expert committees which examine the results at each step of the program. Also, before undertaking a clinical trial, a positive drug must be formulated, and toxicologic and general pharmacologic studies must be completed.

MATERIALS AND METHODS

The experimental data on screening models are stored in the computer files of the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI. Our analysis is based on a Panel Compound Summary that was prepared by the Information Technology Branch of the Developmental Therapeutics Program on July 20, 1981. Because of our particular interest in 69 drugs which have been clinically evaluated, the screening results for these compounds were re-reviewed by the Drug Evaluation Branch, and the Panel Compound Summary for these drugs was updated as of January 12, 1982. Criteria of positivity are those presently in use at the NCI at the confirmatory level (Decision Network Phase 2 [DN2] criteria) for the panel, whereas less strict limits (MC1 criterion) are set for the P388 prescreen. For survival assays, T/C% is computed as the mean (or median) survival time of treated (T) mice divided by that for the controls (C), expressed as a percentage. The MC1 criterion for P388 is T/C% $\geq$ 120. The DN2 criteria for B16, L1210, and LL are T/C% $\geq$ 150. For tumor growth inhibition assays, T/C% is computed as the mean change in weight of treated tumors divided by that for control tumors, expressed as a percentage (for CD8 and Co38, medians are used). The usual DN2 criteria for CD8, Co38, CX-1, LX-1, and MX-1 are T/C% $\leq$ 10.

When studying the relationship between screen results and clinical activity, we considered four tumor sites (breast, head and neck, ovary, and small cell carcinoma of the lung [SCCL]) for which a substantial number of both clinically active and inactive drugs are known. This is because no meaningful correlation study can be done for a tumor site where very few clinically active drugs exist (or for testicular cancer where almost all of the drugs tested are considered active). Although clinical correlations could be investigated meaningfully for only four sites, the clinically active drugs in our analysis include all drugs, with the exception of dacarbazine, known to be active for any solid tumor sites.

The data for single drugs, excluding hormones, published in the medical literature and the files of the Cancer Therapy Evaluation Program (NCI), and of the European Organization for Research on Treatment of Cancer Data Center, were reviewed for clinical effectiveness in advanced cases of solid tumors. A total of 69 compounds were judged to be evaluable for at least one of the four

sites (appendix II). Drugs were considered clinically inevaluable if there was no information in at least one study with 14 or more patients.

In order to overcome arbitrariness in defining clinical activity, two alternative definitions have been used in the analysis. For the minimal definition, a drug is considered clinically active if it produces responses, either complete or partial, in at least 10% of treated patients; 36 drugs met this criterion for at least one of the sites (appendix II). For the strict definition of clinical activity, only drugs known to produce 10% complete responses for at least one of the four sites are considered active, and all other drugs are considered inactive. Only eight drugs met this latter criterion: methotrexate, 5-FU, cyclophosphamide, vincristine, lomustine, cisplatin, doxorubicin, and bleomycin.

When both screening and clinical results are expressed dichotomously, several ratios useful for assessing diagnostic tests (4) can be computed: sensitivity (Sn), specificity (Sp), predictive value of a positive screen (PV+), and predictive value of a negative screen (PV-). Details of computation are given in table 2. For a combination of screens, positivity in at least one of the screens will be regarded as sufficient to attribute positivity to the set. For a screen to be sensible, a drug with a positive screening result must have a higher probability of clinical activity than one with a negative result, thus requiring that $(PV+) > 1-(PV-)$. If this inequality were reversed for a given screen, a random selection of available compounds would be more efficient than the screen for predicting clinical activity.

A fundamental assumption needed to draw unbiased conclusions is that the observed 2 × 2 tables contain random samples of positive and negative drugs for each screen. In fact, the selection of drugs for clinical investigation has been nonrandom, being based on a number of different considerations. The issue of bias in the observed values of Sn, Sp, PV+, and PV- will be discussed in detail below.

TABLE 2.—Contingency table for relating screen results to clinical activity

		Clinic: Active	Clinic: Inactive	
Screen	+	A	B	N1
	-	C	D	N2
		M1	M2	T

A = true-positives
B = false-positives
C = false-negatives
D = true-negatives
PV+ = A/N1
PV- = D/N2

N1 = A + B
N2 = C + D
M1 = A + C
M2 = B + D
Sn = sensitivity = A/M1
Sp = specificity = D/M2

The yield, an additional quantity which does not depend on clinical results, is also useful in assessing the value of a screen. The yield is defined as the ratio of the number of drugs positive in the screen to the total number tested, expressed as a percentage.

RESULTS

Characteristics of the Experimental Systems

The data base consists of 1949 compounds which were tested in at least one of the nine systems. Results were available for the P388 prescreen for 1932 agents, 1635 of which were also studied in at least one of the eight screens of the panel. Data are complete for 866 drugs which were tested in the prescreen, and in all eight screens of the panel. Considering the panel screens only (disregarding P388), 1652 drugs were screened in at least one system, and all eight screens were completed for 867 compounds.

The Prescreen P388

Among the 1635 compounds tested in the P388 prescreen and in at least one of the eight screens of the panel, 1463 (89.5%) were prescreen-positive and 172 (10.5%) were negative, using the MC1 criterion of T/C% ≥ 120. (For most drugs, two P388 values were available, and both had to equal or exceed the cutoff; if only one value was available, it alone was used.) The P388 negative drugs should not have qualified in principle for testing in the panel, but they possessed special features which led the NCI to bypass the prescreen. Of these 172 bypasses, 84 were tested in all eight screens of the panel, of which 13 (15%) were positive (DN2 criterion) in at least one of the eight screens (table 3). Three of these 84 drugs were clinically evaluated. Hexamethylmelamine was positive in the MX-1 screen only; it was evaluated clinically for breast, ovary, and SCCL, and was found moderately active for ovary and SCCL. Baker's antifol and Norgamem were both negative in the prescreen and all eight screens, and were found clinically inactive in breast cancer and head and neck cancer, respectively. The only other prescreen-negative drug that was clinically evaluated was peptichemio, which was found to be positive in L1210 (the only screen for which data were available), but clinically inactive in head and neck cancer.

A cutoff value of 120 had been chosen by the NCI in order to produce only a small number of drugs that were negative in P388 (ie, below the cutoff), but were positive in one or more screens in the panel. These drugs are called "false-negative prescreen" in table 4. This table reveals that increasing the cutoff value to 125 would affect this percentage only slightly, but would importantly decrease the percentage of drugs above the cutoff yet negative in all screens tested (ie, false-positive prescreen).

TABLE 3.—No. of screens in which drugs (tested in all 8 screens) were positive according to the prescreen result*

No. of screens†	P388+			P388-		
	No. of drugs	% of drugs‡	% of drugs§	No. of drugs	% of drugs‡	% of drugs§
0	522	66.8	—	71	84.5	—
1	116	14.8	44.6	11	13.1	84.6
2	71	9.1	27.3	1	1.2	7.7
3	35	4.5	13.5	0	0	0
4	23	2.9	8.8	1	1.2	7.7
5	9	1.2	3.5	0	0	0
6	6	0.8	2.3	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
Total	782	100	100	84	100	100

*MC1 criterion.
†DN2 criterion.
‡% of total (782 for P388+; 84 for P388-).
§% of screen-positive drugs (260 for P388+; 13 for P388-).

This observation applies whether we consider all eight screens, or only screens B16, L1210, and MX-1. This point will be pertinent later. Further increasing the cutoff for P388 might be considered unwise if one wishes to avoid dropping too many drugs at the first stage that would turn out to be positive in the panel. Using 125 rather than 120 as a cutoff would reduce screening costs by about 17% (assuming there are no additional bypasses) because of the substantial number of drugs that would no longer require testing in the panel.

Of those drugs which reached the cutoff of 120 but did not reach 125, three were clinically evaluated. Metoprine was one of these drugs that was found to be clinically active against head and neck tumors. Busulfan and cycloleucine were both evaluated clinically for breast cancer, but were found to be inactive.

The NCI Panel of Eight Screens

None of the drugs tested were positive in all eight screens, as indicated in table 3. Furthermore, no drugs were positive in seven screens, and only six drugs (melphalan, mitomycin, lomustine, semustine, cisplatin, and carmustine) were positive in six of the eight screens. Among the P388 positive drugs tested in all of the screens, 522 (67%) were negative in all eight screens (table 3).

The yield varied greatly from one screen to another (table 5). Only three screens (B16, L1210, and MX-1) had a yield > 10%, whereas Co38, LL, CX-1, and LX-1 were positive for < 4% of the drugs tested. The yield of each individual screen was much less than that produced by using all eight screens together (table 6), where 274 (31.6%) of 867 drugs tested in all eight screens were found to be positive in one or more systems. By examining all possible combinations of screens, it was found that some sets were almost as productive as the whole panel. For instance, using B16, L1210, and MX-1 (screens 1, 4, and 8 in table 6) would uncover 260 (94.9%) of the 274 compounds found by the eight screens (yield of 30.0%).

TABLE 4.—Effect of various cutoff values for the P388 prescreen for predicting whether any subsequent screens will be positive*,†

Prescreen cutoff	All 8 screens		Screens B16, L1210, MX-1	
	% of false+ prescreen	% of false- prescreen	% of false+ prescreen	% of false- prescreen
120	64.5	1.3	66.0	1.0
125	50.6	2.0	51.9	1.6
130	34.9	2.8	36.1	2.2
145	15.7	6.0	16.7	5.2
160	8.6	9.4	9.5	8.4
175	4.0	12.2	4.6	11.0
200	1.6	17.5	2.0	16.1
225	0.6	21.0	0.8	19.4
250	0.2	23.3	0.3	21.5

*For drugs tested in at least 1 of the 8 screens.
†A false+ prescreen drug is positive in P388 and negative in subsequent screens of the panel; a false- prescreen drug is negative in P388 but positive in 1 or more of the subsequent screens of the panel.

TABLE 5.—Positive drugs (yield) and relative cost for each screen of the panel

Screen	No. tested	% positive*	Relative cost (%) vs all 8
B16	1538	14.5	7.8
CD8	1473	6.4	13.7
Co38	1507	2.3	10.2
L1210	1564	14.5	4.4
LL	1493	1.7	9.6
CX-1	1133	2.0	17.1
LX-1	1050	3.3	17.1
MX-1	972	11.9	20.0

* DN2 criterion.

The relative cost of the eight screens also varies considerably (table 5). In particular, CD8 and the three xenograft screens are notably more expensive than the other screens. When taking the cost of each screen into account, the combination of screens B16/L1210/MX-1 would amount to only 32.2% of the cost of all eight screens (table 6).

The final selection of a set of screens depends, however, on the number of clinically active drugs that it will generate and on its clinical predictive value. These ideas will be discussed later.

The Human Tumor Xenografts

The specific contribution of the xenograft screens to the pool of experimentally positive drugs is shown in table 7: 29 drugs were negative in all five homograft screens, but were positive in one or more of the three xenografts of the panel. The mammary model (MX-1) was the most productive of the three screens, since it uncovered 23 of these drugs. Three of the 29 drugs (chlorambucil, hexamethylmelamine, and mithramycin) were clinically evaluated. Chlorambucil was found to be moderately active for cancers of the head and neck and the ovary, and hexamethylmelamine was moderately active for cancer of the ovary and SCCL.

Relationship Between Screen Results and Clinical Activity

Analyses Using the Minimal Definition of Clinical Activity

The values of PV+ have been tabulated by clinical tumor site for each of the eight screens (table 8) and for selected combinations of screens (table 9). The results for L1210 were known for all 69 clinically evaluated drugs. For each of the other screens, there were a small number of incomplete results as indicated in table 8. For the various tabled combinations, the PV+ values are approximately equal within each clinical tumor site, except for the site-oriented combinations (see below). Also, for most of the tabled combinations, the absolute number of clinically active drugs discovered is close to the number found by using all eight screens, and is higher than that obtained by using single screens (compare A, the numerator of PV+, in tables 8 and 9). Specifically, the combination of screens B16/L1210/MX-1 (screens 1, 4, and 8 in table 9) missed only one active drug found by using all eight screens: metoprine was positive in Co38 (but negative in the other seven screens) and was clinically active against head and neck tumors.

An examination of the PV+ of the site-oriented combination for SCCL (LL/LX-1) indicates a somewhat higher

TABLE 6.—No. of drugs tested in all 8 screens positive to at least 1 of the screens in various combinations

Combination of screens*	No. of drugs positive to at least 1 screen	Relative yield (%) vs all 8	Relative cost (%) vs all 8
All 8	274	100	100
1 2 3 4 8	270	98.5	56.1
1 2 4 8	267	97.4	45.9
1 4 8	260	94.9	32.2
1 2 3 4 5	248	90.5	45.7
1 2 3 4	247	90.1	36.1
1 2 4 5	245	89.4	35.5
1 4	230	83.9	12.2
4	145	52.9	4.4
1	140	51.1	7.8

* Key: 1 = B16; 2 = CD8; 3 = Co38; 4 = L1210; 5 = LL; 6 = CX-1; 7 = LX-1; 8 = MX-1.

TABLE 7.—No. of drugs positive in xenograft screens and negative in all the homograft screens of the panel

Screens in which drugs were positive	No. of positive drugs
CX-1 only	2
LX-1 only	4*
MX-1 only	19
CX-1 and LX-1	0
CX-1 and MX-1	1
LX-1 and MX-1	1
CX-1 and LX-1 and MX-1	2
Any of the 3	29

*3 of these were not tested in CX-1 and MX-1.

value reflecting the high PV+ of LL alone. In this case, however, the addition of LX-1 raised the number of clinically active drugs discovered for SCCL from six to 11. One might have hoped that the site-oriented combination of CD8/MX-1 would have a particularly high PV+ for breast cancer, but this was not observed. However, the combination did identify 18 of the 21 drugs clinically active for this site.

There was a modest trend for the drugs which were positive in more screens to be more likely active clinically (table 10). Indeed, for the 15 clinically evaluated drugs which were positive in four or more screens, 12 (80%) were found to be active in at least one of the four clinical sites. On the contrary, of 45 drugs tested in all eight screens but positive in less than four screens, only 21 (47%) were clinically active. Table 11 displays a similar trend for the combination of screens B16/L1210/MX-1. There was also a tendency for higher values of the P388 prescreen to be associated with greater proportions of clinically active drugs (fig 1 and table 12).

Analyses Using the Strict Definition of Clinical Activity

The values of PV+ for each screen and for various combinations are given in table 13. The highest PV+ was found with LL, but this screen missed five of the eight clinically active drugs. The combination of B16/L1210 was as good as any other set of screens and would be sufficient to detect seven of the eight major drugs active, according to the strict definition. There appeared to be some relationship between the number of positive screens and clinical activity according to the strict definition (tables 10,11), which was similar to the results for the minimal definition of activity. The trends were not statistically significant, but the small sample size (only eight active drugs) prevents any firm conclusions. In addition, a relationship was seen between the P388 prescreen result and clinical activity using the strict definition (table 12).

Sensitivity (Sn) and Specificity (Sp)

Considering clinical activity for any site (minimal definition), B16, L1210, and MX-1 were the only screens with observed Sn > 0.50, while Co38, LL, CX-1, and LX-1 had values of < 0.30; CD8 was intermediate. A similar but inverse pattern was seen for specificity. Only Co38, LL, and CX-1 had observed Sp > 0.90, while B16, CD8, L1210, and MX-1 had values of ⩽ 0.72; LX-1 was intermediate.

A high Sn is an important requirement for a primary screen in cancer chemotherapy. Such a screen will uncover a large absolute number of clinically active drugs. The data confirm previous findings that B16, L1210, and MX-1 used together would be a good choice for primary screening. Screens with high Sp are useful as secondary screens in a multistage strategy, because they can reduce the absolute number of false-positives. Co38, LL, and CX-1 fulfill this requirement and could be used for drugs already considered potentially active (because they are positive in any of the three primary screens) in order to decide which drugs are most likely to be clinically effective. As shown in table 14, positivity in any one of these

TABLE 8.—Predictive value of a positive screen (PV+) for each of 4 solid tumor sites using the minimal definition of clinical activity*

Screen	No. of drugs screened†	Breast		Head and neck		Ovary		SCCL		Any site	
		A/N1	PV+	A/N1	PV+	A/N1	PV+	A/N1	PV+	A/N1	PV+
B16	66	13/28	0.46	4/12	0.33	7/14	0.50	10/18	0.55	18/28	0.64
CD8	65	12/22	0.55	6/14	0.43	7/12	0.58	7/15	0.47	16/24	0.67
Co38	64	4/4	1.00	3/4	0.75	2/2	1.00	1/3	0.33	5/5	1.00
L1210	69	16/38	0.42	9/23	0.39	9/18	0.50	12/22	0.55	26/43	0.60
LL	64	6/11	0.55	3/4	0.75	2/5	0.40	6/7	0.86	9/11	0.82
CX-1	67	3/6	0.50	2/4	0.50	3/3	1.00	2/4	0.50	6/7	0.86
LX-1	68	7/16	0.44	2/8	0.25	1/6	0.17	8/12	0.67	10/17	0.59
MX-1	67	14/29	0.48	6/14	0.43	9/13	0.69	12/17	0.71	21/30	0.70
No. of drugs clinically active		21		14		13		16		36	
No. of drugs clinically evaluated		61		35		23		31		69	

*A = No. of drugs positive for a screen that were clinically active for a particular site; N1 = No. of drugs positive for a screen that were clinically evaluated for a particular site.

†No. of drugs (of the 69 clinically evaluated) for which results of the screen were available.

TABLE 9.—Predictive value of a positive screen (PV+) for each of 4 solid tumor sites for various combinations of screens using the minimal definition of clinical activity*

Combination of screens†	Breast		Head and neck		Ovary		SCCL		Any site	
	A/N1	PV+	A/N1	PV+	A/N1	PV+	A/N1	PV+	A/N1	PV+
All 8	21/55	0.38	13/30	0.43	13/23	0.57	16/29	0.55	35/61	0.57
1 2 3 4 8	21/54	0.39	13/30	0.43	13/23	0.57	16/28	0.57	35/60	0.58
1 2 4 8	21/54	0.39	12/29	0.41	13/23	0.57	16/28	0.57	34/59	0.58
1 4 8	21/54	0.39	12/29	0.41	13/23	0.57	16/28	0.57	34/59	0.58
1 2 3 4 5	21/49	0.43	12/29	0.41	11/21	0.52	15/27	0.56	33/55	0.60
1 2 3 4	21/49	0.43	12/29	0.41	11/21	0.52	15/27	0.56	33/55	0.60
1 2 4 5	21/49	0.43	11/28	0.39	11/21	0.52	15/27	0.56	32/54	0.59
1 2 4	21/49	0.43	11/28	0.39	11/21	0.52	15/27	0.56	32/54	0.59
1 4	20/47	0.43	10/26	0.38	11/21	0.52	14/26	0.54	31/52	0.60
2 8	18/36	0.50	—	—	—	—	—	—	—	—
5 7	—	—	—	—	—	—	11/16	0.69	—	—
No. of drugs clinically active	21		14		13		16		36	
No. of drugs clinically evaluated	61		35		23		31		69	

*A = No. of drugs positive for at least 1 screen that were clinically active for a particular site; N1 = No. of drugs positive for at least 1 screen that were clinically evaluated for a particular site.

†Key: 1 = B16; 2 = CD8; 3 = Co38; 4 = L1210; 5 = LL; 6 = CX-1; 7 = LX-1; 8 = MX-1.

three secondary screens identifies a subset of the potentially active drugs having a greater probability of clinical activity. Thus, the results for these secondary screens could be used to set priorities for clinical evaluation of drugs selected by the primary screens.

Proposed Three-Stage Strategy

Our analysis suggested that a modified three-stage strategy (fig 2) might find most of the drugs found by the present two-stage strategy, but at a reduced cost. In the first stage of such a new approach, the P388 model would be used as a prescreen with a result (T/C%) of ≥ 125 on each of two tests considered positive. In the second stage, prescreen-positive drugs would then be tested in the B16, L1210, and MX-1 screens, which have high yield and apparently high sensitivity. Positivity in any one of these screens should be sufficient to consider the drug for clinical trial. In the third stage, compounds that are positive in any of the screens at the second stage can be tested in Co38, LL, and CX-1. Positivity in one of these latter screens, which have apparently high specificity, increases the probability of finding clinical activity. LX-1 and CD8 are not useful in this context since they uncover almost no drugs not already found with the other screens, and do not increase the PV+ of the combination of screens used in the second stage.

If clinical testing had been limited to drugs that met the P388 prescreen cutoff value of 125 and were positive to at least one of B16, L1210, and MX-1, then only three of the 36 clinically active drugs (minimal definition) would have failed to be evaluated clinically. Hexamethylmelamine already failed to meet the present prescreen cutoff of 120; sent to the panel as a bypass, it would have been positive with the new strategy (in MX-1). Bleomycin already failed the present panel, being negative to all eight screens. Only metoprine passed the present strategy but would fail the newly suggested one: it had a

TABLE 10.—Proportion of drugs tested in all 8 screens clinically active for any site by No. of positive screens

No. of positive screens	No. of drugs clinically evaluated	Minimal definition of clinical activity		Strict definition of clinical activity	
		No. of drugs clinically active	Proportion active	No. of drugs clinically active	Proportion active
0-1	22	10	0.45	2	0.09
2-3	23	11	0.48	2	0.09
4-5	9	7	0.78	2	0.22
6-8	6	5	0.83	2	0.33
Total	60	33	0.55	8	0.13
P value for linear trend		0.042		0.104	

TABLE 11.—Proportion of drugs tested in all 8 screens clinically active for any site by No. of positive screens B16, L1210, and MX-1

No. of positive screens	No. of drugs clinically evaluated	Minimal definition of clinical activity		Strict definition of clinical activity	
		No. of drugs clinically active	Proportion active	No. of drugs clinically active	Proportion active
0	9	2	0.22	1	0.11
1	24	13	0.54	2	0.08
2	15	8	0.53	1	0.07
3	12	10	0.83	4	0.33
Total	60	33	0.55	8	0.13
P value for linear trend		0.012		0.122	

minimum P388 value of 123, and was positive only in Co38.

The three-stage strategy proposed here could reduce costs to as low as 36% of the cost of continuing the present strategy, based on two factors: first, only 83% of drugs previously sent to the panel meet the increased prescreen cutoff (assuming there are no additional bypasses); second, rather than testing all drugs in all eight screens, the relative cost of initially using only B16, L1210, and MX-1 and then testing the positive drugs (estimated yield: 30% of those tested) in Co38, LL, and CX-1 is about 43%.

DISCUSSION

To date, the search for curative agents for solid tumors by means of transplanted tumors in mice has been of limited success, since, for a majority of cases, treatment is still mainly palliative. Nevertheless, these limited results are sufficient to demonstrate the usefulness of the transplantable tumor systems, although our analysis suggests that not all of the screens currently in use are necessary. The three-stage strategy proposed in this paper (fig 2) could uncover almost the same number of potentially active new drugs as before, but would be much more economical and faster. The resultant savings should permit the discovery of an increased number of potentially active compounds.

The clinician must be aware of the limitations of predictions based on present tumor models; indeed, one may ask whether any delay in clinical studies imposed on new drugs positive in B16, L1210, or MX-1 for the sake of

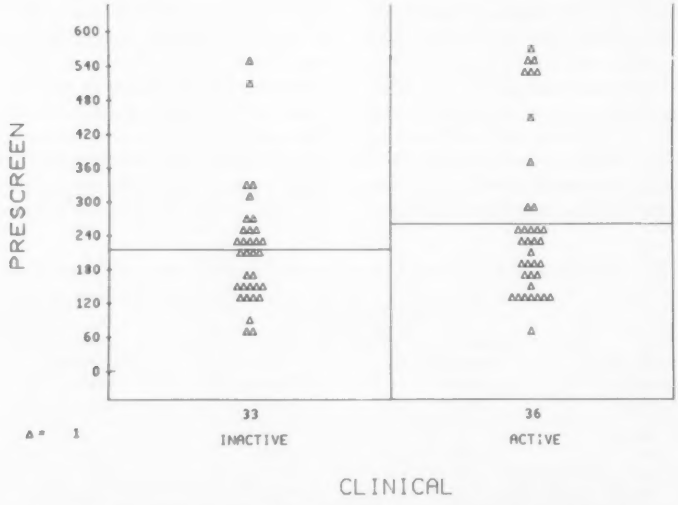

FIGURE 1.—Relationship between minimum P388 prescreen result (T/C%) and clinical activity for any site (minimal definition). Solid horizontal lines represent the means of each group.

TABLE 12.—Relationship between minimum P388 result and clinical activity for any site

Prescreen result	No. of drugs clinically evaluated	Minimal definition of clinical activity: No. of drugs clinically active	Minimal definition of clinical activity: Proportion active	Strict definition of clinical activity: No. of drugs clinically active	Strict definition of clinical activity: Proportion active
< 175	24	10	0.42	1	0.04
175–349	35	18	0.51	4	0.11
350 plus	10	8	0.80	3	0.30
Total	69	36	0.52	8	0.12
P value for linear trend		0.059		0.044	

performing more tests can be justified. As proposed in figure 2, drugs representing new chemical structures of particular promise that show positivity in one of these three screens (ie, in the proposed second stage) could be immediately prepared for phase I–II clinical trials, bypassing the third stage.

To some extent, our analysis confirms the previous observation (5) that drugs active in several different screens are more likely to be effective in humans, but little can be said for a single drug in terms of prediction. The data also suggest that the absolute level of the P388 prescreen may provide an additional basis for ranking drugs. Indeed, in future analyses, one may want to consider the actual numeric values of all screens, rather than just dichotomous responses. Unfortunately, numeric values for the screens were not available to us.

Predictive values considered alone are not sufficient for assessing the value of a screen, because they do not take the yield into account. For instance, CX-1 had a PV+ of 1.0 for ovarian cancer because all three clinically evaluated drugs positive for that screen were clinically active (using the minimal definition). However, this screen, having a low yield (2.0%), failed to detect ten other agents which were also clinically active but negative in the screen. For a primary screen, a high yield, and more importantly, a high sensitivity are the most desirable properties. A screen with such characteristics might thus be preferred to one that happened to have a higher PV+, in spite of the fact that this choice would lead to the evaluation of a larger number of false-positive drugs, because then more clinically active drugs would be discovered.

A difficulty with analyzing clinical results involves the definition of clinical effectiveness in advanced diseases, where an arbitrary percentage of ⩾ 20% of responses (complete plus partial) is customary. In fact, inadequate and variable criteria of response, insufficient sample sizes, differing criteria for selection of patients, and exclusion of so-called inevaluable cases make a precise estimation of response rates unreliable, especially for mar-

TABLE 13.—Predictive value of a positive screen (PV+) for any site for various combinations of screens using the strict definition of clinical activity*

Combination of screens†	A/N1‡	PV+	Active drugs missed§
All 8	7/61	0.11	BLEO
1 4 8	7/59	0.12	BLEO
1 2 3 4 5	7/55	0.13	BLEO
1 2 4	7/54	0.13	BLEO
1 4	7/52	0.13	BLEO
1	5/28	0.18	MTX, 5-FU, BLEO
2	5/24	0.21	MTX, VCR, BLEO
3	1/5	0.20	MTX, CTX, VCR, CCNU, *Cis*-P, ADR, BLEO
4	6/43	0.14	VCR, BLEO
5	3/11	0.27	MTX, 5-FU, VCR, ADR, BLEO
6	1/7	0.14	MTX, 5-FU, CTX, VCR, CCNU, ADR, BLEO
7	3/17	0.18	MTX, 5-FU, CTX, *Cis*-P, BLEO
8	5/30	0.17	MTX, 5-FU, BLEO
2 8 (for breast only)	5/36	0.14	MTX
5 7 (for SCCL only)	4/16	0.25	MTX

*No. of drugs clinically active = 8; No. of drugs clinically evaluated = 69.

†Key: 1 = B16; 2 = CD8; 3 = Co38; 4 = L1210; 5 = LL; 6 = CX-1; 7 = LX-1; 8 = MX-1.

‡A = No. of drugs positive for at least 1 screen that were clinically active for any site (unless otherwise noted); N1 = No. of drugs positive for at least 1 screen that were clinically evaluated for any site (unless otherwise noted).

§BLEO = bleomycin; MTX = methotrexate; VCR = vincristine; CTX = cyclophosphamide; CCNU = lomustine; *Cis*-P = cisplatin; ADR = doxorubicin.

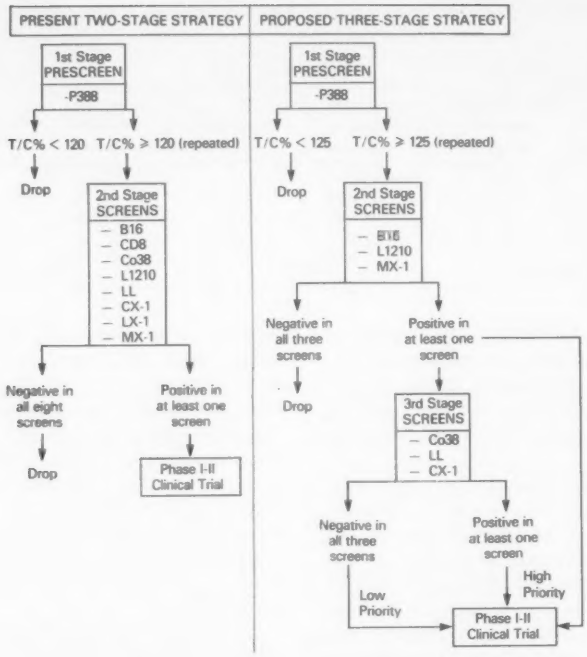

FIGURE 2.—Simplified schema for 2- and 3-stage strategies.

ginally active drugs studied in the past. It is for these reasons that our analysis has been performed using both a strict and a minimal definition of clinical activity, thus covering the possible spectrum of potential effectiveness and removing the need for estimating exactly the percentage of clinical responses in the population of patients. No differences in conclusions resulted when using one or the other definition.

Any assessment of the clinical predictivity of a screening system is limited by definition to drugs for which an evaluation of clinical activity has been performed, and to clinical sites where adequate numbers of both active and inactive drugs are known. It follows that for solid tumor sites where very few clinically active drugs have been identified, any claim that a given screen (new or old) is predictive cannot be substantiated. For these sites, it is not known whether the present screens are inadequate to discover clinically active compounds, or whether few such compounds exist. The situation is different for hematologic malignancies where various active drugs (5-

TABLE 14.—Proportion of drugs (tested in all 8 screens and positive to 1 or more of screens B16, L1210, and MX-1) clinically active for any site by screen result

Screen results		No. of drugs clinically evaluated	No. of drugs clinically active	Proportion active
CD8	+	22	14	0.64
	–	29	17	0.59
Co38	+	4	4	1.00
	–	47	27	0.57
LL	+	10	8	0.80
	–	41	23	0.56
CX-1	+	6	5	0.83
	–	45	26	0.58
LX-1	+	15	9	0.60
	–	36	22	0.61
Total		51	31	0.61

azacitidine, busulfan, thioguanine, cytarabine, daunorubicin, and asparaginase) were either inactive or not tested in the four sites that we studied. A separate analysis would be required to assess screening strategies for hematologic malignancies.

The screening strategy used at the NCI since 1975 was implemented with the purpose of performing a prospective evaluation of clinical predictivity. For solid tumors, such a prospective analysis is not yet feasible, due to the small number of clinically evaluated drugs emanating from this program. Indeed, from 1975 to 1980, 24 Investigational New Drug applications have been filed with the Food and Drug Administration, of which three were dropped after phase I, and three of the 24 are still in phase I evaluation.[7] Clinical data for some solid tumors are available for ten[8] of the 18 drugs currently in phase II clinical trial.

Therefore, in order to reach numbers permitting a meaningful analysis of the correlation between mice and humans, it was necessary to use the data on all clinically evaluated drugs, of which 59 (85%) were not uncovered through the present strategy. In analyzing the data as if these 59 drugs had been found using the present NCI strategy, it was seen that more clinically active drugs were positive in L1210 than in the other screens. This finding does not demonstrate that the L1210 screen is highly predictive, but reflects only the historical predominance of this model in selecting drugs for clinical evaluation.

The unbiased, prospective evaluation of the clinical predictivity of a screen, or a combination of screens, would require that random samples of all drugs, both screen-positive and screen-negative, be studied clinically. The nonrandom sampling of the drugs actually selected for clinical trial can therefore introduce bias into the results. Indeed, drugs negative in all known experimental systems are not selected for phase I–II clinical trial, because the concept of using such compounds is not ethically acceptable by clinicians. In addition to biased selection of drugs for clinical evaluation based on screening results, other factors prevent or discourage the execution of phase I–II studies with all screen-positive drugs, ie, the high cost of synthesis, insufficient supply, difficulties in formulation, unacceptable toxicity, chemical analogy with existing drugs, and lack of clinical investigators.

In order to investigate the extent of possible bias in the four quantities Sn, Sp, PV+, and PV– when they are computed from the observed 2 × 2 tables, it is informative to compare them with theoretic results based on all screened drugs (whether or not they have been clinically evaluated). Unbiased, theoretic values could be calculated by constructing 2 × 2 tables (defined in table 2) using the actual figures for N1, N2, and T (N1/T is the yield of the particular screen), and choosing a range of sensible values for M1 (the total No. of clinically active drugs) and A (the No. of these active drugs that are positive for a given screen). Note that M2, B, C, and D are thus automatically defined. This theoretic exercise has suggested that the observed values of Sn and PV+ are likely too high and values of Sp and PV– are likely too low.

If we now consider how the observed tables are generated, we can rationalize these findings. Because the process that selects drugs for clinical testing favors positive screening results, an observed table represents an oversampling of the top row of the theoretic table, that is, oversampling of screen-positive drugs. If the oversampling had been randomly performed, there would be no bias in the estimation of PV+ or PV–, but Sn would be overestimated and Sp would be underestimated. However, there are reasons to believe that the selection process is better than random and that it tends to identify drugs that are more likely to be clinically active. For example, we have shown that the probability of clinical activity was related to the number of screens in which a drug was positive. This means that the observed table represents an oversampling from the left column, as well as the top row of the theoretic table. The likely result is that PV+ is too large, PV– too small, Sn too large, and Sp too small. Unfortunately, the extent of these biases depends on the unobservable true values of M1 and A, and therefore, no corrections of the observed ratios seem reasonable. The observed PV+ values are nevertheless descriptively useful in examining various screening strategies. Observed PV– values are not reported here because consideration of possible sampling biases has suggested that these values are less likely to be correct than values for PV+. Observed Sn and Sp have been used only to suggest a division of the screens into groups with high and low values.

Our analysis was limited to the eight screens in the NCI panel, and the results provided to us were based on the established DN2 criteria. Other experimental models have been developed and new ones will continue to be proposed. These may differ from those in the panel in many ways, ie, origin of the tumor, specifics of transplantation or cell culture, tumor burden involved, and criteria of response. The rationale for the development of new experimental models is based on increasing the clinical predictivity of the screens (3,6). As new screens that are designed to relate better to the clinical situation are investigated, it is important that the results be analyzed appropriately. Our analysis shows the value of considering the results of screens in relation to one another. More importantly, the effects of biased sampling that we have discussed should be kept in mind.

[7] S. Marsoni, personal communication (Aug 1981).

[8] Anguidine, pyrazofurin, maytansine, zorubicin, chlorozotocin, PALA, amsacrine, PCNU, bruceantin, and mitoxantrone (July 1981).

APPENDIX I.—Characteristics of tumors comprising the prescreen and the 8 individual screens in the NCI Tumor Panel

Tumor	Code	Origin of tumor	Host of origin	Histologic description	Test host	Site
			Mouse tumors			
Leukemia (prescreen)	P388	Chemically induced with methylcholanthrene	DBA/2 mouse	Lymphocytic leukemia	BDF1 or CDF1	Ip
Melanoma	B16	Spontaneous at base of ear	$C_{57}B1/6$ mouse	Melanoma	BDF1 or B6C3	Ip
Breast	CD8	Spontaneous	$CD8F_1$ mouse	Mammary adenocarcinoma	CD8F1	Sc
Colon	Co38	Induced by chemical carcinogen, 1,2-dimethylhydrazine	$C_{57}B1/6$ mouse	Colon adenocarcinoma	BDF1	Sc
Leukemia	L1210	Chemically induced with methylcholanthrene	DBA/2 mouse	Lymphocytic leukemia	BDF1 or CDF1	Ip
Lung	LL (Lewis)	Spontaneous in lung	$C_{57}B1/6$ mouse	Carcinoma	BDF1	Iv
			Human tumor xenografts			
Colon	CX-1	Human colon	Isolated in tissue culture, subsequent maintenance in nude mice	Colon adenocarcinoma	Nu/Nu Swiss	SRC*
Lung	LX-1	Human lung	Isolated in nude mice	Carcinoma	Nu/Nu Swiss	SRC
Breast	MX-1	Human breast	Isolated in nude mice	Infiltrating duct cell carcinoma	Nu/Nu Swiss	SRC

*Subrenal capsule.

APPENDIX II.—Clinical activity of 69 drugs for 4 solid tumor sites according to the minimal definition of activity*

Drug	Abbreviation	NSC No.	Tumor site			
			Breast	Head and neck	Ovary	SCCL
Methotrexate†	MTX	740	1	1	1	1
Busulfan	BUS	750	0	NT	NT	NT
Thioguanine	6-TG	752	0	NT	NT	NT
6-Mercaptopurine	6-MP	755	0	1	0	NT
Mechlorethamine	HN_2	762	1	0	1	1
Cycloleucine	CYLEU	1026	0	NT	NT	NT
Dactinomycin	ACT.D	3053	0	NT	1	NT
Chlorambucil	CHL	3088	0	1	1	NT
Thiotepa	THIOT	6396	1	NT	1	NT
Metoprine	DDMP	7364	NT	1	NT	NT
Melphalan	L-PAM	8806	1	NT	1	1
Triethylenemelamine	TEM	9706	0	NT	NT	NT
Hexamethylmelamine	HXM	13875	0	NT	1	1
5-Fluorouracil†	5-FU	19893	1	0	1	0
Mithramycin	MITHRA	24559	0	NT	NT	NT
Norgamem	NOR	25855	NT	0	NT	NT
Cyclophosphamide†	CTX	26271	1	1	1	1
Mitomycin	MITO-C	26980	1	NT	NT	0
Floxuridine	FUDR	27640	1	0	1	NT
Hydroxyurea	HYD	32065	NT	1	NT	0
Mitoguazone	Me-GAG	32946	0	1	NT	NT
Emetine	EME	33669	NT	NT	NT	0
Trimethylcolchicine	TMCA	36354	NT	0	NT	NT
Streptonigrin	STNI	45383	0	0	NT	NT
Dacarbazine	DTIC	45388	0	0	NT	NT
Vinblastine	VLB	49842	1	1	1	0
Porfiromycin	POR	56410	NT	1	NT	NT
Azotomycin	AZO	56654	0	0	NT	NT
Chromomycin-H_3	CH-H_3	58514	0	NT	NT	NT
Cytarabine	ARA-C	63878	0	NT	NT	NT
Vincristine†	VCR	67574	1	NT	0	1
Procarbazine	PCZ	77213	0	0	NT	1
Lomustine†	CCNU	79037	1	0	0	1
TIC-mustard	TIC-HN_2	82196	1	NT	NT	NT

APPENDIX II.—Clinical activity of 69 drugs for 4 solid tumor sites according to the minimal definition of activity*

Drug	Abbreviation	NSC No.	Tumor site			
			Breast	Head and neck	Ovary	SCCL
Streptozocin	STZ	85998	0	NT	NT	0
Dibromomannitol	DBM	94100	0	NT	NT	NT
Semustine	Me-CCNU	95441	0	NT	0	1
PCNU	PCNU	95466	0	NT	NT	NT
Yoshi-864	YOS	102627	0	0	1	0
5-Azacitidine	5-AZA	102816	0	NT	NT	NT
Mitolactol	DBD	104800	1	1	NT	1
Cytembena	CYTB	104801	0	0	NT	NT
Ifosfamide	IPH	109724	1	NT	NT	1
Cisplatin†	CIS-P	119875	0	1	1	0
Teniposide	VM26	122819	0	0	NT	NT
Doxorubicin†	ADR	123127	1	1	0	1
Bleomycin†	BLEO	125066	0	1	NT	0
Razoxane	ICRF-159	129943	0	0	NT	0
Dianhydrogalactitol	DAG	132313	0	0	NT	0
Piperazinedione	PIP	135758	NT	NT	0	NT
Baker's antifol	BAF	139105	0	NT	NT	NT
Anguidine	ANG	141537	0	NT	NT	NT
Etoposide	VP16-213	141540	0	0	0	1
Pyrazofurin	PYR	143095	0	0	NT	0
Cyclocytidine	CYCLO-C	145668	0	NT	NT	NT
Tegafur	FT-203	148958	1	0	NT	NT
Maytansine	MAY	153858	0	0	NT	0
Zinostatin	NCS	157365	0	NT	NT	NT
Zorubicin	RUB	164011	0	NT	NT	NT
Bruceantin	BRU	165563	0	NT	NT	NT
A.ara-5-fl.cyt.	AAFC	166641	0	1	NT	1
Chlorozotocin	CLZ	178248	0	NT	NT	0
Carubicin	CAR	180024	1	NT	0	NT
PALA disodium	PALA	224131	1	NT	NT	NT
Vindesine	VDS	245467	1	0	NT	1
Peptichemio	PTC	247516	NT	0	NT	NT
Amsacrine	*m*-AMSA	249992	0	NT	0	0
Mitoxantrone HCl	MITOX	301739	1	NT	NT	NT
Carmustine	BCNU	409962	1	NT	0	1

*1 = active, 0 = inactive, NT = not tested.
†Active according to the strict definition in 1 or more sites.

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The National Cancer Institute Antitumor Drug Discovery Program, Current and Future Perspectives: A Commentary[1]

John M. Venditti[2,*]

This issue of *Cancer Treatment Reports* includes a report by Staquet et al (1) entitled "Clinical Predictivity of Transplantable Tumor Systems in the Selection of New Drugs for Solid Tumors: Rationale for a Three-Stage Strategy." The study is based on 5-year (1976–1981) results from the National Cancer Institute (NCI) in vivo preclinical "tumor panel experiment" composed of a prescreen, mouse leukemia P388, from which active compounds were selected for screening, and a panel of five mouse tumors: leukemia L1210, melanoma B16, mammary tumor of $CD8F_1$ mice (CD), colon 38 (C8), and Lewis lung carcinoma (LL), and the three human tumor xenografts, mammary (MX-1), colon (CX-1), and lung (LX-1). The report assesses the clinical predictability of the tumor panel by comparing screening results with efficacy against human cancers of breast, head and neck, ovary, and lung (small cell carcinoma) for 69 drugs, including 64 which were tested clinically prior to use of the preclinical panel.

Much has been written about the tumor panel since its inception, because of the importance of choosing the best preclinical models in the search for better anticancer drugs, and also because the availability of this massive contract-generated data base provided unprecedented opportunities to address critical questions. Most prior dissertations, including our report (2), discussed the tumor panel within the context of the overall antitumor drug development program of the NCI, or analyzed "preclinical-clinical correlations" retrospectively, using available but insufficient data from both settings. Our previous report (2) concentrated on findings, other than the screening results, that would impact on the development of improved methods for discovering better anticancer drugs.

As far as we are aware, the paper by Staquet is the first statistically based study which used nearly all of the screening results from the tumor panel. The authors deserve commendation for their hard work and sense of responsibility to the screening program; they spent many hours with us clarifying data points, as well as points of view. We have completed our own analysis (3) of results from the tumor panel from 1976 to 1982,[3] and have implemented a three-step sequential screening plan (table 1) very similar to that proposed by Staquet with respect to the first two steps, and substantially different in the third step. For this reason it is appropriate to compare the two studies (1,3) relative to objectives, conclusions, and recommendations. The purpose of this commentary is to present our current screening practice and some future plans relative to antitumor drug discovery. Our analysis of panel results, per se, was the major factor impelling current operational changes in the screening program (table 1). However, other significant findings that emerged during the development and conduct of the panel (2), results of other contract-supported projects (2,4), and recent advances in basic research have significantly influenced our overall approaches to the discovery of new antitumor drugs.

At the outset of the tumor panel experiment, we recognized the impracticality of screening many thousands of compounds each year against eight tumors. It was necessary to devise a means of selecting compounds for screening in addition to intellectual selection based on structures (5) and pattern recognition (6). Although we would test any material with known biologic or biochemical action suggestive of potential antitumor selectivity against the entire panel (2), it seemed sensible to subject the bulk of the compounds to a relatively sensitive and inexpensive in vivo prescreen; those compounds showing a modest effect would become candidates for screening against the tumor panel. The P388 leukemia was chosen as the prescreen for reasons that have been previously discussed (2). The low end point (increase in life span $\geq$ 20%) was purposeful in order to minimize false-negative predictions for panel activity. Staquet's analysis shows that P388 accomplished this objective. Our study (3) emphasizes the value of P388 by showing a strong direct corre-

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[3]Our preliminary analysis was presented to the Division of Cancer Treatment (DCT) Board of Scientific Counselors in Feb 1981, and was updated for the Board in June 1982.

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TABLE 1.—Flow of drugs through NCI preclinical in vivo screens, 1983*

Step	Estimated input of compounds/yr†	Tumor(s)	Tumor site	Treatment site	Time of treatment initiation
1—Prescreen	10,000	P388	Ip	Ip	Day 1
2—Modified panel	250	L1210	Ip	Ip	Day 1
		B16	Ip	Ip	Day 1
		MX-1	Src‡	Ip	Day 1
		M5076	Ip	Ip	Day 1
3—Secondary screening §	50	L1210	Sc	Ip	Day 1
		B16	Sc	Ip	Day 1
		B16	Ip	Sc	Day 1
		M5076	Sc	Ip	Day 1
		MX-1	Sc	Ip	When sc tumor is established
		CD	Sc	Ip	When sc tumor is established
		C8	Sc	Ip	Day 1
Treatment of established (advanced) tumors shown sensitive to early treatment					
Determination of profile of cross-resistance to major clinical drugs					
Antimetastatic screens					
Other assays as indicated by prior found activities, structure, etc					

*Summarized from Venditti et al (ref 3).

†Estimates from experience with 1976–1982 tumor panel.

‡Tumor implanted under kidney capsule (subrenal capsule) (ref 2).

§Specific assays for each drug selected on basis of prior responsiveness. It should be noted that routine route and schedule-dependency testing and the bioassay of experimental dose formulations are conducted for drugs in development to clinical trial independently of this screening plan.

lation between the quantitative level of prescreen activity and the probability of activity in the panel. For example, we found that of compounds producing an increase in life span (ILS) of 20%–29% in mice with P388, 5% of the compounds were active in one or more of the tumor panel assays. Of those compounds producing an ILS of 50%–69% against P388, 37% were active in the panel. Eighty-four percent of compounds eliciting an ILS ≥ 100% against P388 were active in the panel. The gratifying performance of P388 indicates that it should be retained as a prescreen, and reinforces the notion that very good anticancer drugs are generally effective against a number of tumors. These anticancer drugs, however, act via a limited number of mechanisms, and the use of P388 alone as a prescreen limits the potential to discover and develop different types of compounds which may have unique actions. The use of more than one in vivo assay in the prescreen is precluded because of costs, and our future plans include the development of in vitro assays. In developing the tumor panel concept in 1975 (7), we observed that in vitro assays amenable to large-scale screening lacked the specificity required to select for in vivo therapeutic efficacy. However, in 1981 (2) we noted significant advances in cell culture technology, especially in cloning human tumor cells from fresh surgical explants. We initiated an ambitious program to modify existing procedures (which had been developed to test clinically effective drugs whose tolerable concentrations in humans were known as an aid in selecting treatment for individual patients), and to extrapolate them to the large-scale screening of essentially unknown materials. Quality-controlled protocols for screening in human tumor clonogenic assays have been developed and applied to the screening of 79 compounds which had been inactive against P388 in vivo, and several compounds with novel structures have demonstrated outstanding in vitro activity (4). Such in vitro testing may be an effective way of identifying compounds which would otherwise be lost to the screening program. We learned, however, that the complexities and costs of human tumor colony-forming assays using fresh surgical explants precluded the testing of more than a very small percentage of P388 negative compounds. Therefore, we are hoping to develop simpler in vitro assays which may be suitable for large-scale prescreening, and which are based on the use of serially transferable human tumor cell lines, especially those which retain functions characteristic of their tissue of origin.

Staquet points out that the program could realize a modest cost saving and lose relatively few tumor panel active drugs by raising the ILS required for prescreen activity from 20% to 25%. The point is well taken, but the manner of adjusting the prescreen end point (if a change is indicated) depends on a number of more complex factors. At present, for a material to "pass" the prescreen, an ILS of ≥ 20% must be confirmed. The principal ineffi-

ciency in present practice is that there is some range of activities with limits below and above 20% within which differences are not significant. Thus, a relatively high percentage of materials which attain the 20%–25% ILS level in the first test do not "confirm." One might set the end point for the initial P388 test at some higher, experimentally observed level indicative of a 95% probability that the true ILS is ⩾ 20%. We are currently using our historical P388 data to analyze retesting and compound reacquisition efficiency and costs, as a function of the end point for activity in the initial test.

An examination of our analysis of tumor panel results (3) will show agreement with Staquet with respect to the "yield" of active[4] compounds from individual assays or groupings of assays.[5] For example, both Staquet and our study (3) found that 95% and 94% (respectively) of compounds active in at least one panel assay were active against L1210, B16, or MX-1. In our study, only 13 of 225 panel-active compounds failed in all three of these assays, and each of the 13 was active against only one of the five remaining tumors. We also agree with Staquet that the cost of testing against L1210, B16, and MX-1 was about one-third the cost of testing against the entire panel (the estimate was based on cost figures supplied by us) and that it is not cost efficient to screen all prescreen actives against the remaining tumors.

The tumor panel experiment was designed to maintain a steady flow of new agents for development to the clinic and to provide information relative to the following three questions:

a. To what extent would broad spectrum screening select new agents that would have been missed by using only L1210, historically the most prevalently (sometimes exclusively) used in vivo assay?

b. Do human tumor xenografts and mouse tumors select the same or different drugs?

c. Do human tumor xenografts and mouse tumors of a specific histology or tissue of origin select the same drugs?

Because of the emphasis on discovering new agents, addressing specific questions that would influence future screening practice, and providing preclinical results that would facilitate a prospective assessment of preclinical-clinical correlations, our analysis (3) excluded tumor panel results for 12 clinical compounds and their congeners: doxorubicin, dactinomycin, cisplatin, cyclophosphamide, 5-FU, cytarabine, bleomycin, methotrexate, mechlorethamine, nitrosoureas, mitomycin, and vincristine. This exclusion of current clinical drugs represented a fundamental difference from the analytical approach of Staquet, whose judgments about clinical predictability relied heavily on a largely retrospective comparison of tumor panel results for drugs with prior known clinical efficacy. As these authors have pointed out, it is not possible to reach very firm conclusions from such analyses for all but a few clinical malignancies, because of the small number of effective drugs for many human solid tumors, and because of the lack of uniformity of criteria for clinical activity, especially for older drugs. We would add that retrospective studies may be biased seriously when the preclinical arm of the study includes a selected set of drugs which may have previously entered the clinic because of activity against a preclinical tumor under evaluation. Thus, in our forthcoming analysis (3), we offer no conclusions regarding predictability, preferring to await clinical feedback for new agents. Finally, there is the problem of comparisons using different units of measurements, eg, increase in life span, or tumor inhibition in the preclinical setting and response rate in clinical trials.

The tumor panel did uncover new active compounds which were not active against L1210. Of 1085 compounds that were tested against all panel tumors, 106 were active against L1210. Of the 979 compounds that were inactive against L1210, 119 were active against one of the "solid" tumors, 78 against B16, 45 against MX-1, 18 against CD, ten against LX-1, nine against CX-1, five against C8, and one against LL. Table 2 lists 30 compounds for which applications to conduct investigational clinical trials were filed with the Food and Drug Administration from January 1977 through March 1983, and an additional seven compounds for which filing is anticipated by the end of 1983. The listing does not include all drugs for which an application was filed, but is restricted to drugs for which experimental antitumor activity was an important factor in prompting development to clinical trial. Activity in one or more tumor panel assays was instrumental in the decision to go forward with 33 of the drugs listed in table 2. Thirteen of the 33 were not active against L1210. An additional 25 drugs, 13 of which were not active against L1210, are in various earlier stages of development to clinical trial.

We noted earlier that of 225 compounds with activity

[4]The screening program has established two efficacy values as benchmark criteria for each in vivo assay: a lower end point indicative of statistical significance (MC-1 activity), and a higher end point indicating sufficient antitumor selectivity to warrant consideration of the agent for presentation to the DCT Decision Network (DN) Committee as a candidate for development to clinical trial (DN activity). Staquet and our study (3) both use the term "activity" to indicate DN level activity for the eight tumor panel models. For the P388 prescreen, however, "activity" is used to indicate attainment of the lower end point (MC-1) in order to minimize false-negative predictions for tumor panel activity. The MC-1 and DN activity criteria for each assay have been previously discussed (2).

[5]Agreement is expected because the data base used in both studies was nearly the same. Staquet had available data for 1652 compounds which had been tested in at least one of the panel assays, and for 867 compounds which had been tested in all eight assays. Our analysis (3) was based on data for 1085 compounds, with testing complete in all eight assays. We excluded results for 12 known clinically effective drugs and their close structural congeners, for reasons explained in the text. The results for these compounds were a prominent factor in the analysis by Staquet. Our exclusion of clinical drugs and their analogs also explains why, in some instances, the number of "active" compounds reported by our group is smaller than the number reported by Staquet, despite our larger data base.

TABLE 2.—INDAs filed or anticipated during 1977–1983 based on preclinical antitumor activity*

NSC No.	Name	Responsive tumors†
	Active against L1210	
51143	Pyrazolo (2,3-a) imidazoline (IND closed)	*L1210*
153353	L-alanosine	*L1210*
169780	ICRF-187	*L1210*
322921‡	Bisbenzimidazole	*L1210*
325014‡	Bactobolin	*L1210*
139490	Emolfin sodium; 5-methyltetrahydro-homofolic acid	*L1210*, methotrexate-resistant L1210
286193	Tiazofurin	*L1210*, *LL*
312887	Fludarabine phosphate; 5-fluoro-ara-AMP	*L1210*, *LX-1*
337766	Bisantrene	*L1210*, *B16*
249992	*m*-AMSA	*L1210*, *B16*, *CD*
296934	Teroxirone	*L1210*, *B16*, *CD*
269148‡	Menogarol	*L1210*, *B16*, *CD*
301739	Mitoxantrone	*L1210*, *B16*, *MX-1*, *C8*
95466	PCNU	*L1210*, *B16*, *MX-1*, *CD*, *LL*, *LX-1*, mouse tumors implanted intracranially
163501	Acivicin	*L1210*, *MX-1*
182986	AZQ	*L1210*, *MX-1*
264880	Dihydro-5-Azacytidine	*L1210*, *MX-1*
280594	Triciribine phosphate	*L1210*, *MX-1*, *CD*
7365	DON; 6-diazo-5-oxo-L-norleucine	*L1210*, *MX-1*, *LX-1*
218321	Pentostatin; deoxycoformycin	Potentiation of arabinosyl adenine activity by inhibition of adenosine deaminase
404241	9-Arabinosyl adenine	*L1210* when combined with adenine deaminase inhibitors
	Active against B16 or MX-1; not active against L1210	
165563	Bruceantin	*B16*
526417	Echinomycin	*B16*
305884	Acodazole	*B16*
325319‡	Didemnin B	*B16*
352122‡	Trimetrexate	*B16*
224131	PALA	*B16*, *CD*, *LL*
132319	Indicine-*N*-oxide	*B16*, *MX-1*
125973‡	Taxol	*B16*, *MX-1*
241240	Carboplatin	*B16*, *MX-1*, *CD*
172112	Spiromustine; spirohydantoin mustard	*B16*, *MX-1*, *CD*, colon 26, IC-EM§
253272‡	Caracemide	*MX-1*
118742	Pentamethylmelamine	*MX-1*, human lung xenograft (not LX-1)
3051	*N*-methylformamide	*MX-1*, *CX-1*, *LX-1*
	Not active against L1210, B16, or MX-1	
141633	Homoharringtonine	*C8*
21548	Thymidine	Human melanoma xenografts
208734	Aclarubicin	Active against P388, reported to be less toxic than doxorubicin (Japan)

*During this period, INDAs were filed for 10 additional materials which are not listed, because the bases for development to clinical trial were not pertinent to the evaluation of these screening models. The materials include radiation modifiers, analgesics, antiemetics, and reported clinical activity.

†Tumor panel assays are shown in italics.

‡INDA not yet filed, but anticipated during 1983.

§An intracranially implanted mouse ependymoblastoma.

in one or more tumor panel models, only 13 were inactive against L1210, B16, or MX-1. Each of the 13 compounds was active against only one of the remaining five tumors. The Investigational New Drug application (INDA) has been filed for one compound, homoharringtonine, which is active against C8 only. A flavone derivative, NSC-347512, also active against C8 only, is in an early stage of development.

Our analysis (3) shows that 81 compounds were active against one or more of the three human tumor xenografts in the panel, and 26 of these, including 22 MX-1 actives, were inactive against all five mouse tumors. INDAs were filed for pentamethylmelamine, which is active against MX-1 only, and *N*-methylformamide, which is active against all three xenografts, but none of the mouse tumors. Filing for caracemide, which is active against MX-1 only, is expected this year.

We found the degree of correlation between responses of human and mouse tumors of similar tissue origin to be no greater than correlations when the response of a human tumor derived from one tissue was compared with the response of a mouse tumor of a different type (3). However, this apparent lack of correlation based on tumor type may be misleading. The tumor panel included one mouse tumor and one human tumor of each of three types: breast, colon, and lung. We had previously discussed (2) the influence of cellular heterogeneity on relative responsiveness to chemotherapy among tumors of a given type, and pointed to abundant evidence that tumors of similar tissue origin do not respond to drug treatment uniformly. The use of multiple human-animal tumor pairs of a given type in the tumor panel experiment was precluded by cost considerations. However, the development of in vivo xenografting and in vitro cloning capabilities for a wide spectrum of human tumors has provided technology that may permit a proper preclinical study of the relationship of tumor type to therapeutic responsiveness. More importantly, it may be possible to express preclinical activity as response rate, the parameter used most often to measure clinical efficacy.

This year, we plan to implement a project for developing preclinical screens based on sequential in vitro and in vivo testing against batteries of human tumors, a battery consisting of ten or more tumors of a specific histologic type. The objectives are to determine the extent to which in vitro assays, using transplantable human tumors grown in athymic (nude) mice as the source of tumor cells, are capable of identifying drugs with activity against the same tumors in vivo and the development of preclinical screens that permit expression of response rate against specific human cancers. The moderate to low "evaluability rate" for fresh surgical explants in the clonogenic assay (4) will not be a drawback, since the tumors included in each battery will be chosen because of their ability to be established as transplantable tumors in athymic mice, and because of their demonstrated adequate and reproducible cloning efficiency when taken from any transplant generation. We are cognizant of the potential for loss of drugs requiring metabolic activation in vivo, but this can be overcome by relatively simple in vitro technique modifications. The principal advantages of this approach are the following:

a. It is disease-oriented rather than drug-oriented; that is, the goal is to find drugs for clinical trial against those cancers with the best chance to respond.

b. Expression of preclinical findings in terms of percentage responders against a specific type of cancer will permit more valid preclinical-clinical correlations, because results of broad clinical trials are most often expressed in this way.

c. A positive correlation between in vitro and in vivo responsiveness of tumors will make this approach more cost-feasible, because it eliminates the need for expensive in vivo testing against individual tumors that do not respond in vitro.

Step 1 (P388 prescreen) and step 2 (abbreviated tumor panel) of our current in vivo plan (table 1) are identical to stages 1 and 2 as proposed by Staquet with one exception: we tentatively plan to add mouse tumor M5076 to L1210, B16, and MX-1 (table 1, step 2), because preliminary evidence suggests that M5076 is a high-yield assay, and thus, may uncover agents not found by the other three assays. Also, M5076 exhibits highly specific patterns of metastases and can be inhibited by macrophage activating agents (8–10), characteristics of potential usefulness in studies of antimetastatic potential, and mode of action of the drugs to which it responds. Our current plan is to test a reasonable number of new materials against M5076, and to retain it in the new panel if it identifies active agents not found by L1210, B16, or MX-1.

Step 3 of the plan outlined in table 1 is quite different from stage 3 as proposed by Staquet. The latter stipulates the use of C8, LL, and CX-1, three refractory tumors, because of their "apparently high specificity." More might be gained in this step by reducing the number of very refractory tumors, eliminating very expensive and nonresponsive xenografts, and concentrating on detailed studies of active drugs in a way that is likely to produce more useful information for establishing priorities for development and clinical use. We have included one relatively refractory mouse tumor, C8, in step 3 (table 1), mainly because it has unique sensitivity to homoharringtonine and the flavone derivative.[6] We included

[6] If the current plan (table 1) had been operational when these compounds were screened, and if progression to step 3 required attainment of DN level activity (5) in step 2, these "sole C8 actives" would not have been tested against C8. Perhaps progression to step 3 should be based on attainment in step 2 of the MC-1 end point, or some end point lower than the DN requirement. Homoharringtonine was active against L1210 and B16 and NSC-347512 was active against B16, but these activities failed to meet DN criteria.

CD in step 3 because of its moderate sensitivity. In examining panel activity for 36 drugs which met the minimal definition for clinical activity established by Staquet, we found six drugs active against CX-1 and ten drugs active against LL. Thirteen of these 16 drugs were active against CD. Of the eight drugs that met Staquet's strict definition for clinical activity, all that were active against CX-1 or LL were also active against CD; indeed, seven of the eight drugs were active against CD. One might argue against our decision to delete low-yield and expensive assays from routine screening on the basis that the few compounds active against only these tumors may be very important clinically. It is important to keep in mind that each tumor is in the DCT frozen tumor bank and can be regenerated in vivo if future clinical feedback should indicate a special desirable attribute. In the meantime, it would seem advantageous to suspend the use of such assays in favor of channeling resources into other experiments which are likely to be more enlightening. Since the main objective of step 3 (table 1) is to provide a maximum of preclinical information for active compounds, we chose to emphasize studies of the influence of modifying factors on activities such as route of drug administration relative to tumor implant site, body burden of tumor cells, and patterns of cross-resistance to clinical drugs. Thus, step 3 (table 1) is designed to permit use of simple laboratory manipulations to increase the challenge presented to the newly found antitumor agent. The specific assays and experimental designs to be used in step 3 are to be selected on the basis of the known activities of the drug, rather than in conformance to a rigidly prescribed protocol. At this step in the flow of compounds through the preclinical screening system, the survivors are few and deserve intensive care.

The selection of past and present antitumor screens has been based on retrospective preclinical-clinical correlations, feasibility for large-scale screening, unusual patterns of responsiveness, biologic principles of chemotherapy from more fundamental studies, and attempts to answer critical questions such as those presented to the eight-tumor panel. Given the need to continue to find new effective anticancer drugs and the economics of preclinical screening, we can but apply the most pertinent scientific information available to the selection of compounds for screening and to the selection of test assays as well.

Past changes in the size and constituency of the input of compounds and the test assays, as well as the new projects described in this commentary, exemplify the responsiveness of the NCI preclinical drug development program to emerging scientific advances. While past and present practices have had a major impact on improvements in cancer treatment and curability, recent significant advances in molecular biology, medicinal and organic chemistry, pharmacology, and biochemistry provide unprecedented opportunities for the design and preclinical evaluation of new potentially powerful anticancer therapies. Exploitation of these leads and their extrapolation into new drugs with unique mechanisms of action and new strategies for cancer treatment will require mobilization of the most talented and dedicated scientists. Therefore, we plan to implement National Cooperative Drug Discovery Groups (NCDDG).[7] It is anticipated that each NCDDG will be multidisciplinary and multi-institutional; that is, it will be composed of the most creative scientists in academic, nonprofit research, and industrial organizations. The goal of the NCDDG program is the application of strong scientific rationales to the design and synthesis of new anticancer therapies, and to the selection or development of the most informative preclinical assays for the recommendation of new treatments for human cancer. The NCDDG program will be conducted concomitantly with the existing drug screening and evaluation program. The two programs are envisioned as being mutually complementary.

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Doxorubicin in Pregnancy: Possible Transplacental Passage[1]

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Two pregnant women with lymphoproliferative disorders were treated with doxorubicin-containing regimens. Both patients delivered shortly after a dose of doxorubicin. One child was healthy and the other was stillborn. Measurements of anthracycline levels in placental, cord, and fetal tissues by high-performance liquid chromatography suggest that doxorubicin may be transported across the placenta. [Cancer Treat Rep 67:773–777, 1983]

The occurrence of cancer in pregnant women is relatively uncommon. In the past, one approach to management was to perform a "therapeutic" abortion if the pregnancy was in the first two trimesters. The reluctance to carry a pregnancy to term was often based on the assumption that treatment of the mother with chemotherapy would be deleterious to the fetus. In addition, little was known regarding the fetal-maternal pharmacology of the commonly used antineoplastic agents. Recently, however, advances in chemotherapy and perinatology have allowed the continuation of pregnancies during administration of chemotherapy to the mother. Numerous reports document the subsequent delivery of normal infants (1–10).

Doxorubicin and other anthracycline antibiotics are particularly active in leukemias, lymphomas, and breast cancer (11), neoplasms which often present during the childbearing years. The current report describes the measurement of anthracycline levels in fetal and placental material from two pregnant women who went into labor soon after receiving a dose of doxorubicin.

CASE REPORTS

Patient 1

A 30-year-old $G_2P_0Ab_1$ woman presented with symptoms of fatigue, myalgias, and low back pain during her 21st week of pregnancy. Previous medical history was unremarkable except for a pregnancy that ended at 15 weeks with a spontaneous abortion. Physical examination revealed mild pallor, a 2/6 systolic ejection murmur, moderate thoracic scoliosis, and uterine enlargement consistent with a 21-week gestation. Wbc count was 2600/mm^3 with a differential including 7% lymphoblasts. Hematocrit was 29.9% and platelet count was 111,000/mm^3. A bone marrow biopsy established the diagnosis of acute lymphoblastic leukemia. Induction therapy consisted of doxorubicin, vincristine, and prednisone. The patient achieved complete remission without apparent significant toxic effects to her or to the fetus. Fetal development was carefully followed with the aid of periodic ultrasound examinations. Subsequently, intensification therapy was begun with doxorubicin, vincristine, prednisone, L-asparaginase, intrathecal methotrexate, and cranial irradiation.

During her 34th week of pregnancy, and 48 hours after receiving 45 mg/m^2 of doxorubicin (total cumulative dose, 214 mg/m^2), the patient's membranes ruptured spontaneously and she went into labor. Because of a footling breech presentation, a cesarean section was performed. A vigorous 2080-g male infant was delivered with Apgar scores of 8 at 1 minute and 10 at 5 minutes. The child's physical examination was normal, including a full head of hair. A cbc of the neonate was normal, with a hematocrit of 71%, a platelet count of 241,000/mm^3, and a leukocyte count of 6700/mm^3 (with a differential of 75 segmented neutrophils, 22 lymphocytes, two monocytes, one metamyelocyte, and two nucleated rbcs). An echocardiogram was normal. Chromosomal analysis was not performed. Thirty months after delivery the child has continued to develop normally and the mother has remained in complete remission after completion of maintenance chemotherapy.

The placenta, cord, and cord blood were analyzed for doxorubicin and its metabolites.

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Patient 2

A 28-year-old $G_3P_2Ab_0$ woman presented at 25 weeks' gestation with 1 month of progressive dyspnea. Physical examination revealed severe tachypnea, signs of superior vena cava obstruction, lymphadenopathy at the thoracic inlet, and uterine enlargement consistent with a 25-week pregnancy. Routine laboratory studies and a bone marrow biopsy were normal. A chest x-ray demonstrated massive mediastinal adenopathy with tracheal compression. Lymph node biopsy was not performed because of superior vena cava obstruction. Therapy was promptly initiated with dexamethasone and radiotherapy consisting of 900 rads in 3 fractions over 36 hours. There was dramatic resolution of the mediastinal mass and the patient's symptoms. Fetal dosage from radiation scatter was estimated to be between 9 and 18 rads.

An abdominal ultrasound at 26½ weeks' gestation was normal, with estimated fetal weight at the 25th percentile. At 30 weeks, while the patient was receiving tapering doses of dexamethasone, the mediastinal mass recurred, along with a pleural effusion and a breast mass. Biopsy of the breast mass established a diagnosis of diffuse, undifferentiated lymphoma of T-cell origin. At 31 weeks, chemotherapy was begun with doxorubicin (45 mg/m^2), vincristine, and prednisone.

Fetal cardiac activity was confirmed by ultrasound on the day of chemotherapy. However, the mother had last noted fetal movement the previous night. Thirty-six hours after treatment, a repeat ultrasound demonstrated intrauterine fetal death. Laminaria were inserted into the cervix in preparation for induction. The patient subsequently went into spontaneous labor and precipitously delivered a 1200-g stillborn female infant 60 hours after the administration of chemotherapy.

Although the fetus was macerated, an autopsy revealed no additional fetal abnormalities. The placenta was immature, with several small areas of recent infarction. There was extensive endothelial damage, organizing thrombosis, and occlusion and recanalization of the chorionic vessels.

Fetal tissues were subsequently analyzed for the presence of anthracycline compounds.

MATERIALS AND METHODS

Analysis of Doxorubicin and Metabolites in Tissues

Freshly obtained samples of placenta and fetal tissues were washed in ice-cold saline, blotted dry, and either processed immediately or frozen and stored at -20°C for subsequent analysis; if frozen, samples were thawed on ice before processing. Samples were weighed and covered with 9 volumes of Tris-HCl buffer [0.05 M, pH 8.5, containing 3% sodium dodecylsulfate (wt/vol)]. Tissues were minced, then homogenized in a ground glass tissue grinder in the presence of the Tris buffer. Homogenates were extracted three times with 2 volumes of ethyl acetate:*n*-propanol (9:1, vol/vol). To the pooled extracts of each tissue *N*-trifluoroacetyladriamycin-14-octanoate (AD 28) was added as internal standard, and the extracts were evaporated to dryness in a vortex evaporator. The residues were reconstituted in 200 μl of methanol and centrifuged. The supernatants were aspirated and stored at -20°C for 15 minutes to allow residual sodium dodecylsulfate to separate out. A 20–25-μl aliquot of the clear methanolic extract was used for analysis by high-performance liquid chromatography (HPLC). The analytic conditions employed (Waters Associates μ-Bondapak/phenyl column, acetonitrile-pH 4.0 ammonium formate buffer programmed development, flow fluorescence detection) have been previously described (12); lower limits of 50 pmols/g of tissue and 20 pmols/ml of plasma or serum are readily detectable and quantifiable for doxorubicin and doxorubicinol by this methodology. Recovery of doxorubicin and doxorubicinol by the extraction procedure is > 70% from plasma, and > 50% from tissues, as determined from studies with mice (13).

Recovery and Characterization of Unknown Fetal Metabolite

The methanol-reconstituted extracts from all the tissues in Patient 2, as examined by HPLC, were pooled. The combined extract was spotted as a streak on a glass-backed silica gel thin-layer chromatography plate (250-μm layer) (Analtech, Wilmington, DE). Development of the plate was with chloroform-methanol (95:5, by volume). After being air-dried, the plate was examined for fluorescence under 254- and 350-nm light. Two fluorescent bands were observed; these were scraped off the glass, and the fluorescent materials were extracted from the silica gel into methanol. As determined by HPLC, one of these materials was the AD 28 internal standard which had been added to each of the tissue extracts; the other material was the unknown having the 5.26-minute retention time. The fluorescence emission spectrum of the unknown was determined in methanol on a Perkin-Elmer Model MPF-4 spectrofluorometer at an excitation wavelength of 484 nm.

RESULTS

Patient 1

Doxorubicin levels in placental tissue and cord blood are shown in table 1. The highest levels of doxorubicin were recorded in placental tissue distant from the umbilical cord. Lower levels were noted in the placenta proximal to the cord. Doxorubicin was detectable at a low level in the umbilical cord itself, but none could be found in cord blood. No metabolites were detected.

TABLE 1.—Doxorubicin levels: Patient 1

Sample	Doxorubicin (nmols/g of tissue)
Placenta (maternal side)	1.186
Placenta (proximal to cord)	0.786
Umbilical cord	0.083
Cord blood plasma	Not detectable

Patient 2

No doxorubicin could be detected in any fetal tissue (table 2). However, an HPLC signal common to all of the tissues was seen at a retention time of about 5.26 minutes (some variation in retention time is normal over long periods of sampling time). The retention time of this material does not correspond to any previously characterized doxorubicin metabolite. The greatest such signal was detected in the fetal spleen. The fluorescence emission spectrum of this unknown, determined after separation by preparative thin-layer chromatography, was essentially identical to that of doxorubicin and other anthracycline materials having the intact daunorubicin or doxorubicin chromophore.

DISCUSSION

Concern over the use of anticancer drugs during pregnancy stems from the assumption that such drugs may be teratogenic to the human fetus. Animal experiments clearly demonstrate that susceptibility to a teratogen varies with the developmental stage of the fetus. Current data suggest that the same may be true for humans (14,15). Drugs reported to be deleterious to the human fetus include aminopterin (16), cyclophosphamide (17,18), chlorambucil (19), busulfan (20–22), methotrexate (23), 6-mercaptopurine (24), and 5-FU (25).

Anthracyclines have been demonstrated to elicit chromosomal aberrations in human leukocytes (26,27). They are mutagenic (28) and carcinogenic (29–31) in vitro and in vivo in animals. Their effects on the human fetus, however, remain unknown. We are unaware of any reports of abnormalities in fetal development in humans following anthracycline therapy during pregnancy. Premature delivery has been observed (1,2,5,32), but it is impossible to eliminate the role of other drugs or of the underlying cancer in these cases. Two reports (10,33) document the use of doxorubicin in the first trimester without adverse effects on the fetus.

The pharmacokinetics of doxorubicin are complex and probably reflect its interactions with diverse cellular constituents. Plasma levels decrease rapidly at first, followed by a slower decline with a half-life of about 24 hours (34,35). Excretion is primarily via the biliary route (36). Intracellular levels often greatly exceed plasma concentrations (37). With respect to pregnancy, it is crucial to know at what rate and in what form doxorubicin might reach the fetus. In the only previous study of the transplacental passage of doxorubicin, Roboz et al (38) could not detect the drug or its major metabolite in amniotic fluid shortly after a dose was administered to the mother.

Levels of anthracyclines have now been measured directly in placental and fetal samples after doxorubicin administration to the mother. The assay procedure utilized a well-established high-sensitivity liquid chromatographic methodology originally developed in our laboratories. While daunorubicin is usually used as the internal

TABLE 2.—Doxorubicin levels in fetal tissues: Patient 2

Fetal tissue	Doxorubicin (nmols/g of tissue)	Other metabolites	
		Retention time (mins)	nmols/g*
Liver	ND†	1.04‡	0.115
		5.32	0.335
Lung	ND	5.26	0.295
Kidney	ND	5.28	0.400
Heart			
Left	ND	5.25	0.288
Right	ND	5.25	0.241
Spleen	ND	5.14	0.944
		5.30	1.975
Diaphragm	ND	5.32	0.276
Duodenum	ND	5.34	~0.060
Skeletal muscle	ND	5.33	0.251

* Expressed as AD 28 equivalents.

†ND = not detectable.

‡Fluorescence signal emerging at solvent front presumably due to anthracycline and/or aglycone conjugate (normally seen in liver).

standard in this system, for the present study a different standard, one eluting sharply with a late retention time (8.60 minutes) distinctly clear of any known doxorubicin metabolites or breakdown products, was selected so as to avoid possible masking by the internal standard of any chromatographic region of interest. Because the AD 28 standard may be acted on by esterases present in plasma and tissue homogenates, it was necessary to add the internal standard to the organic extracts of the samples, rather than to the samples prior to extraction. From previous studies it is known that AD 28 is fully stable and provides adequate quantitation of doxorubicin and metabolites when used in this way.

Placental levels of doxorubicin in Patient 1 were measured approximately 48 hours after a drug bolus. The greater level seen in the maternal side of the placenta possibly could be explained by the fact that this area is partially composed of some decidual (ie, maternal) tissue. The identification of doxorubicin in the umbilical cord itself is noteworthy since the cord derives its blood supply solely from the cord blood. Thus, one must postulate that some of the drug had reached the fetal circulation previously but was below the limits of detection at the time it was measured.

Fetal tissues of the second patient revealed no detectable levels of doxorubicin or known metabolites. However, the HPLC signal detected at 5.26 minutes may represent a previously uncharacterized doxorubicin derivative. This signal is not attributable to any internal standard breakdown product since the AD 28 is completely stable under the assay conditions; furthermore, the signal was present in scouting runs with samples to which no internal standard had been added. The fluorescence emission curve of the unknown showed a profile typical of the anthracycline fluorochrome and was essentially superimposable on that of doxorubicin. The fact that the highest levels of this compound were recorded in the spleen further suggests that it is derived from doxorubicin: previous work in mice has demonstrated that doxorubicin tends to accumulate in the spleen (M. Israel and V. K. Khetarpal, unpublished observations). No "normal" fetal tissue was available to serve as a control. However, no similar signal has ever been seen in any of our studies with tissues derived from animals not treated with anthracyclines.

In addition to the usual metabolic products (doxorubicinol and 7-deoxyaglycones), doxorubicin is known to decompose to give 6, 8, 11-trihydroxy-l-methoxy-5, 12-naphthacenedione (39). Reference samples of this material (provided by Dr. L. Malspeis, Ohio State University) showed retention times of 5.48 minutes under the assay conditions used in this study, a value significantly different from the range of retention times found for the unknown in the fetal tissue samples. Also, the naphthacenedione compound has a fluorescence emission spectrum different from that of doxorubicin, thereby further eliminating it as a possible structure for the unknown.

In Patient 2, the extensive vasculopathy of the chorionic vessels is unusual. The presence of organizing thrombosis and recanalization suggests that these lesions occurred a long time prior to the administration of doxorubicin. Whatever physiologic process led to fetal death is unlikely to be related to the chemotherapy given to the mother.

Although the data are not conclusive, they suggest that doxorubicin or its metabolites may cross the placenta and reach the fetus. More detailed studies are required to better characterize doxorubicin's transplacental passage and to evaluate its effect on fetal growth and development.

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Acute Liver Dystrophy With Thrombosis of Hepatic Veins: A Fatal Complication of Dacarbazine Treatment[1]

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In the course of adjuvant single-agent chemotherapy with dacarbazine after extirpation of malignant melanoma, a fatal complication was observed in four of our patients. During the second cycle of therapy liver failure due to massive hepatic necrosis supervened, with widespread thrombosis of liver veins. The clinical and pathologic features of our patients are compared with the findings in eight published cases. The possible pathogenesis is discussed in detail, and Budd-Chiari syndrome caused by an allergic thrombophlebitis with secondary liver cell necrosis seems to be most probable. [Cancer Treat Rep 67:779–784, 1983]

Dacarbazine (DTIC) is a cytostatic drug which has been used recently in cancer chemotherapy. In dermatology it has been applied lately as adjuvant single-agent chemotherapy for malignant melanoma. Known side effects have been neutropenia and thrombocytopenia, nausea, vomiting, mild functional disturbances of liver, and a flu-like syndrome (18). Since 1979, an additional fatal complication has been attributed to DTIC therapy: acute liver failure caused by extensive liver necrosis and acute thrombosis of liver veins (3,4,11,13,14,22,24,25).

The purpose of the present paper is to report in detail four new cases, comparing the clinical course and the morphologic findings with the already published cases, and to analyze the formal and causative pathogenesis.

CASE REPORTS

Patient 1

A 59-year-old female caucasian presented with a superficial spreading melanoma (level III; thickness, 1.70 mm) which was excised from the right flank using local anesthesia (300 mg of mepivacaine and 0.273 mg of epinephryl). She received tetracycline (500 mg), *o*-rutoside (600 mg), metildigoxin (0.3 mg), sulfamethoxazole (1600 mg), and trimethoprim (320 mg) postoperatively. Thirty-seven days after surgery a combination of single-agent chemotherapy and immunotherapy (250 mg/m^2 = 425 mg of DTIC daily for 5 days and 0.01 ml of BCG intracutaneously on the 6th day) was started. She was taking metoclopramide (10 mg) as an antiemetic.

Laboratory values were normal before starting therapy. The patient did not tolerate the cytostatic therapy very well, and on the third day her temperature rose to 38°C. The patient recovered quickly after the cessation of the first cycle of therapy. The second cycle of chemotherapy started after 17 days. The transaminase activity was slightly elevated (SGPT, 30 units/L, GGT, 40 units/L) at this time. Furthermore, the eosinophilic granulocyte count rose to 19.7% (total wbc count, 10.4 × 10^6/L). The body temperature was 39°C on the evening of the second day of the second cycle of DTIC treatment and was normalized only by acetaminophen (1000 mg). The DTIC treatment was accordingly interrupted (total dose, 3010 mg). On the evening of the third day shaking chills developed. A circulatory shock developed during the following night. The circulation could be stabilized temporarily by dopamine and the usual shock treatment. However, the patient died 34 hours after the initial signs of shock. In the postmortem liver biopsies and at necropsy extensive fresh hemorrhagic necrosis of liver parenchyma and fresh thrombosis of sublobular veins were seen. The walls of patent

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and occluded veins were infiltrated intensively by eosinophilic granulocytes, lymphocytes, and plasma cells. Similar inflammatory infiltrates were seen in the portal triads. As a sign of terminal bacteremia, intravascular gram-positive bacilli could be found microscopically. The vena hepatica communis, the vena cava and their branches, and the portal vein were patent. Arteriosclerosis of coronary arteries was pronounced but not stenosing. Metastases of the malignant melanoma or epithelioid granulomas were not present.

Patient 2

A 57-year-old caucasian had a skin lesion excised at the buttocks, which was not examined histologically. After 4 months a swelling of the right groin appeared; operative resection performed under halothane anesthesia revealed a malignant melanoma in lymph nodes. Adjuvant DTIC chemotherapy (400 mg daily for 5 days) combined with oral BCG and transfer-factor intracutaneously was started 22 days after this operation. Metoclopramide (10 mg), dimenhydrinate (300 mg), diazepam (5 mg), and ascorbic acid were administered daily. Laboratory values were nearly normal at the beginning and end of the first therapeutic cycle (total wbc count, 11.7 × 10^9/L; eosinophilic granulocyte count, 2%; SGOT, 5 units/L; SGPT, 3 units/L; GGT, 9 units/L; and erythrocyte sedimentation rate, 2/6 (the Westergren method). Thirty days later the second DTIC cycle was started (400 mg daily). On the second day eosinophilia (eosinophilic granulocyte count, 19%; wbc count, 9.8 × 10^9/L) was disclosed.

After the second injection of DTIC the patient vomited frequently. After the third application of DTIC (total dose, 3200 mg) the vomiting increased, and after about 14 hours a circulatory shock developed simultaneously with epigastric pain. The liver was palpable 15 cm below the right costal margin and was firm and tender. Two days after the first symptoms of shock a slight neutrophilia (11,700 cells/mm^3) developed and the transaminase activity was raised considerably (SGOT, 4360 units/L; SGPT, 4840 units/L). After circulation conditions improved, acute renal failure occurred with an oligo-anuric phase and then a polyuric phase. Furthermore, subileus and disseminated intravascular coagulation were seen. Eight days after the shock the patient died, with the clinical picture of a terminal sepsis.

At necropsy, the liver showed extensive hemorrhagic, partially anemic, necrosis of parenchyma of different ages. Some necroses were fresh, and others were demarcated by granulocytes; some necrotic cells were already phagocytized. Small to large liver veins were occluded partially by thrombi in early organization. Vein walls contained erythrocytes and were infiltrated slightly by lymphocytes and neutrophilic granulocytes. The vena hepatica communis, the vena cava and their branches, and the portal vein were patent. Portal triads were infiltrated sporadically by lymphocytes, plasma cells, and granulocytes, and bile ductuli were proliferated. Due to the portal hypertension an ascites of 2000 ml had developed. Granulomatous lesions caused by treatment with BCG or metastases of the malignant melanoma were not detected. As a sign of shock, "shock-kidney," a necrotizing ischemic enterocolitis of esophagus and large bowel as well as isolated necrosis of pancreas and fat-cells, was found. Death was caused by liver and heart failure.

Patient 3

A 59-year-old male caucasian presented with a nodular malignant melanoma (level III) which was excised at the back; he had noticed the tumor 4–5 weeks previously. Nine days after surgery, DTIC (400 mg daily for 5 days) was given in combination with metoclopramide (10 mg) and meclizine (50 mg) in addition to immunotherapy with BCG drinking ampules (three daily) and human transfer-polyvalent factor (100 ml). The laboratory values, especially transaminases and wbc counts, were normal. After 24 days the second DTIC cycle was started (400 mg daily). At the same time metoclopramide (10 mg), meclizine (50 mg), and diazepam (10 mg), as well as 2 × 1 bottles of human transfer-factor, were administered. After the second injection of DTIC, the patient's axillary temperature increased to 38.9°C. The temperature normalized after 3 days but increased again to 38.9°C after further application of DTIC (total dose, 3200 mg). The fever was treated symptomatically by drops of metamizole. On the fourth day of the second therapeutic cycle with DTIC, acute abdominal pain and tension occurred, which was treated by infusions of physiologic saline, dextran 60, cafedrine (1000 mg), and theodrenaline (50 mg). The patient died about 4 hours later with the presumptive clinical diagnosis of acute cardiac infarction with normal laboratory values. At necropsy, the liver showed pronounced blood congestion (with local nutmeg appearance) and necrotic hepatocytes (with partial parietal and obturating thrombosis of small and medium-sized hepatic veins). The thrombosis disclosed beginning organization and might have been 2–3 days old. The walls of liver veins were intensely infiltrated by eosinophils, a moderate number of lymphocytes, and sparse neutrophils and plasma cells. The portal triads showed similar inflammatory infiltration. Central veins, big hepatic veins, vena hepatica communis, vena cava and their branches, and the portal vein were patent and without thrombi. Granulomas were not obvious. Malignant melanoma metastases were not detected. In addition to a general arteriosclerosis, a stenosizing arteriosclerosis of the coronary arteries was found with an old throm-

botic occlusion of the left interventricular branch and a 4 × 4-cm scar on the left ventricular wall. The patient probably died in acute cardiac failure.

Patient 4

The clinical symptomatology and the morphologic findings of this 50-year-old caucasian have already been reported in detail (22). The excision of superficial spreading melanoma under halothane anesthesia was followed by single-agent therapy (DTIC) combined with immunotherapy. The patient died during the second cycle of therapy 24 hours after the second application of DTIC (total dose, 3500 mg) with signs of liver failure which was caused by Budd-Chiari syndrome (BCS) with thrombotic occlusion of small to great hepatic veins. An allergic genesis of liver vein phlebitis was presumed.

TABLE 1.—Clinical data

	No. of patients
Female	5
Male	7
Total	12
Mean age in yrs: 54.8 ± 6.5	12
Mean total dose of DTIC in mg (range): 3066.4 ± 683.2 (1750–4030)	11*
No. of doses of DTIC before onset of shock (during 2nd cycle of DTIC treatment)	
2	7
3	4
4	1
Mean time interval between start of 2nd cycle of DTIC treatment and death (days): 3.29 ± 2.95	12
Mean time interval between tumor excision and shock (days): 85.4 ± 90.7	10*

*Data unavailable for remaining patients.

DISCUSSION

The clinical course and morphology of the liver observed in our four patients are in good agreement with the eight cases already published (tables 1-4). Until now, nausea, vomiting, slight functional liver disturbances, and bone marrow depression have been described as side effects of DTIC treatment (18). Since 1979, extensive liver cell necrosis and thrombosis of hepatic veins have been ascribed to single-agent DTIC chemotherapy in several case reports (3,4,11,13,14,22, 24,25). It is interesting that there are published reports of two cases of extensive liver cell necrosis and thrombosis of hepatic veins which occurred during multiple-agent chemotherapy including DTIC (15,16); this fatal complication occurred, as with our patients, during the second cycle of therapy. In two additional cases, the possibility of a relationship between death with widespread liver necrosis and multi-agent chemotherapy (including DTIC) is more difficult to determine owing to the use of halothane for anesthesia shortly before the DTIC treatment (23). Clinical observations indicate that complications developing after DTIC treatment, similar to those noted here, are not as rare as was previously supposed (9,10,14). The morphologic findings described for the liver have been commented on variously with regard to pathogenesis. On the one hand it has been interpreted as the sequel of a shock, triggered by the hepatotoxic action of DTIC or by one of its metabolites (25). The other hypothesis is that thrombotic lesions of

TABLE 2.—Additional clinical data: liver function

Assay	No. of patients*		Level of increased activity
	Normal activity	*Increased activity*	
Alkaline phosphatase (before treatment)	9	1	192 units/L
SGPT (between Cycles 1 and 2)	4	4	29.3 ± 3.7 units/L
SGOT (during Cycle 2)	0	9	4005.9 ± 3778.4 units/L
Eosinophils	3	6	11.5 ± 4.9%
	Absent	*Present*	
Hb_SAG	2	1	
Acute viral hepatitis	1	—	
Fever	1	7	

*Data unavailable for remaining patients.

TABLE 3.—Morphologic findings

Liver pathomorphology	No. of patients* Absent	Present
BCS	—	11
Thrombophlebitis of liver veins	2	9
Thrombophlebitis of liver veins with eosinophil infiltration	4	5
Malignant melanoma metastases	6	4

*Data unavailable for remaining patients.

hepatic veins developed primarily as BCS, possibly as a result of an allergic vasculitis with secondary liver cell necrosis by congestion (22) (fig 1).

From the present cases it is still difficult to establish the most likely pathogenesis of this fatal complication during DTIC chemotherapy and to answer the questions raised above. We shall, however, discuss in detail the pathogenetic possibilities outlined above.

The first hypothesis (ie, of a purely toxic pathogenesis) is improbable, since the total applied dose of the cytostatic drug DTIC was 3198 ± 568.2 mg (n = 10), lower than the 4500–7500-mg dose of DTIC usually applied during adjuvant chemotherapy. The possibility that an abnormal sensitivity to the cytostatic drug might have existed in these patients cannot be excluded with certainty. Other factors could have a synergistic and promoting effect. On the one hand it is possible that other drugs, eg, antiemetics, interfere with the catabolism of DTIC. However, no such synergistic drug reaction is stated in the literature, nor have comparable lesions of the liver been attributed to these drugs (12). Moreover, experiments have shown that iv injected BCG decreases the *N*-demethylation of DTIC in the microsomes of the liver (17). However, BCG immunotherapy in four of ten patients does not seem to have essentially influenced the course of the disease.

TABLE 4.—Additional therapy

Therapy	No. of patients Treated	Data unavailable
Anesthesia		
Local	1	5
General	1	
Halothane	5	
2-3 halothane	2	
Time interval between halothane and start of chemotherapy: 62.8 ± 8.5 days		
Antiemetics*	9	2
Other drugs†	7	3
Immunotherapy with BCG	5	2
Transfer factor	2	2
Blood tranfusions	0	10

*Metoclopramide, triflupromazine, dimenhydrinate, meclizine, haloperidol, chlorpromazine, and amitryptiline.

†Diazepam, oxazepam, trimethoprim, sulfamethoxazole, tetracycline, *o*-rutoside, methyldigoxin, estrogen, and metamizol.

Furthermore, a causal relationship between halothane anesthesia and fatal outcome seems improbable because of the long symptom-free interval (62.8 days) compared to about 21 days usually observed in this rare idiosyncratic drug reaction (2). Additionally, cancer by itself might slow down the metabolism of a cytostatic drug, especially if the tumor is widespread (17), which could have been effective in only two cases.

Moreover, to our knowledge, thrombosis of liver veins has never been described in the acute phase of intoxication with unequivocal liver toxins (eg, carbontetrachloride). The possibility that the shock which occurred in DTIC-treated patients had been induced by drug toxicity can also be further excluded on the grounds that the morphologic pattern of shock liver is quite different from the findings described above (21). Finally, the morphologic features were not typical of a fulminant hepatitis, which can be differentiated diagnostically; thrombosis of liver veins has been found more frequently in fatal subacute hepatitis than in fulminant hepatitis (19).

For the above reasons the thrombotic occlusion of hepatic veins seems to be the primary event with a secondary destruction of liver cells through congestion as in BCS. BCS can be caused by different factors or basic diseases. The etiology can be clarified exactly in no more than about 30% of all patients (20). BCS is rarely found at autopsy (0.05%–0.1%). This expected value is slightly exceeded by the observed frequency of 0.22% of BCS during DTIC therapy at the University of Cologne in 1979 and 1980 (No. of all autopsies, 893). The frequent coincidence of the rare BCS and the DTIC chemotherapy suggests a causal relationship and not a merely accidental finding. The clinical course with the dramatic fatal complication at the beginning of the second therapeutic cycle 24 hours after the second or third dose, after an interval without therapy, suggests a hyperergic pathogenesis. The allergic reaction seems to be provoked during the second cycle of DTIC after the induction phase of the first cycle. This hypothesis is supported by the thrombophlebitis of liver veins (n = 7), with inflammatory infiltration by abundant eosinophils (n = 5). It may be argued in favor of this explanation that eosinophilia could be detected in > 40% of patients (n = 28) treated with DTIC, without occurrence of that fatal complication (22). Finally, reports about photosensitivity and urticarial reactions demonstrate the allergic potency of DTIC. An immune complex disease appeared to be most probable as the underlying type of immune reaction (5,7,26). In recent years BCS has been described after therapy with some cytostatic drugs (mercaptopurine, 6-thioguanine, cytarabine, urethane, and azathioprine) (2,6). The disease developed after a far longer cytostatic therapy

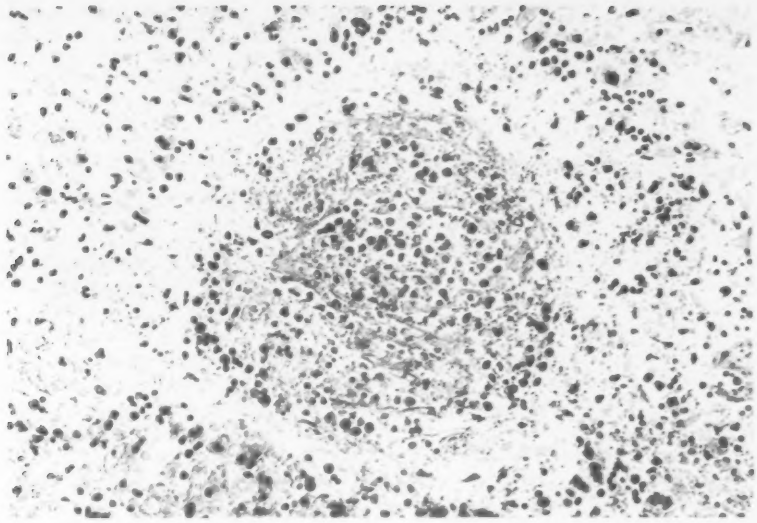

FIGURE 1.—Endophlebitis of a smaller hepatic vein with infiltration by abundant eosinophils and a moderate No. of neutrophilic granulocytes, lymphocytes, and plasma cells and thrombosis; hematoxylin and eosin stain, × 160.

compared with the cases treated with DTIC. The vascular lesions corresponded morphologically to the so-called veno-occlusive disease of the liver after intoxication by pyrrolizidin-alkaloids (1,8). It is very probable that the BCS developing during therapy with the above-mentioned cytostatic drugs is due to toxic effects. There is a significant difference between, on the one hand, the smouldering clinical course after extended cytostatic therapy with a high cumulative dose together with the morphologic condition of veno-occlusive disease and, on the other hand, the incidents which occurred, as described, during the DTIC treatment. This comparison makes it very probable that we are dealing with an allergic event provoked by DTIC.

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Phase I Study of Iv Mycobacterial Cell Wall Skeleton and Cell Wall Skeleton Combined With Trehalose Dimycolate[1,2]

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A phase I study to determine the toxicity and immunologic effects of iv administered aqueous suspensions of mycobacterial cell wall skeleton (CWS) and CWS combined with trehalose dimycolate (TDM) was performed. A total of 26 patients received suspensions of either CWS or CWS/TDM given iv every 1 or 2 weeks in set doses of either 100, 250, 500, or 1000 $\mu g/m^2$. The major dose-limiting side effects for both preparations were fever and chills at a dose of 1 mg/m^2. This was more severe with the combined CWS/TDM. There was no renal or pulmonary toxicity. There was evidence of hepatic toxicity, with increases in the alkaline phosphatase and gamma glutamyl transferase, especially with high doses of CWS/TDM. Both the CWS and CWS/TDM groups had an increase in their wbc count. In the patients receiving CWS alone, there was a decrease in the peripheral blood monocytes. In the CWS/TDM group, there was an increase in the serum lysozyme, suggesting macrophage activation. No antitumor effect was seen in this phase I study. Aqueous suspensions of CWS and CWS/TDM can be administered safely iv over a period of time, with evidence of an effect on hematologic parameters. [Cancer Treat Rep 67:785–790, 1983]

Nonspecific immunostimulation with mycobacterial agents, especially BCG, has been widely utilized in an effort to increase host resistance to malignancy (1). Crude viable and/or killed mycobacterial agents are heterogeneous preparations which have a significant incidence or morbidity (2,3). Because of these factors of heterogenicity and toxicity, major interest has centered on the purification and use of microbial components.

In the guinea pig, oil-attached mycobacterial cell wall skeleton (CWS) and trehalose dimycolate (TDM) given intralesionally is as effective as viable BCG in causing tumor regression and systemic immunity (4,5). We have demonstrated similar results using these materials intralesionally in patients with malignant melanoma (6,7). The potential use of systemic iv microbial agent is appealing from the standpoint of ease of administration, lack of local toxicity, and the potential for inducing systemic effects. Iv BCG itself has been utilized in man (8). However, most studies in this area have involved *Corynebacterium parvum* or the methanol extracted residue (MER) of BCG. *C. parvum* has undergone extensive trials in both animals and man (9). Given iv, it had demonstrated activity against pulmonary metastasis in mice (10) and visceral metastasis in man (11).

MER is an effective agent in augmenting immunologic responses (12–15). MER, used intralesionally or sc, has demonstrated antitumor activity in animals when used as a single agent or in combination with chemotherapy or irradiation (16,17). MER has also demonstrated limited antitumor activity in man (18). The systemic iv use of MER has recently been evaluated in dogs (19) and in man (20,21). Delipidated mycobacterial cells and purified cell walls in saline are preparations similar to MER which are effective against Ehrlich carcinoma and lymphoid leukemia in mice when given ip (22).

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MATERIALS AND METHODS

Preparation of Microbial Components

CWS from *Mycobacterium smegmatis* was prepared as previously described for BCG CWS (4). TDM was purified by microparticulate chromatography from cord factor of *M. tuberculosis*, Agoma B. strain.

Two preparations for iv use were prepared. An aqueous suspension of CWS was prepared by homogenizing *M. smegmatis* CWS (Lot BS III K) at 1 mg/ml in Dulbecco's phosphate-buffered saline containing 0.2% Tween 80. The material was then irradiated to 10,000 rads to ensure sterility. The resulting material was stored at room temperature. For clinical use the appropriate dose was diluted in 100–500 ml of normal saline and infused iv over a period of 30 minutes.

The second preparation consisted of a suspension of equal amounts of CWS (Lot BS III K) and TDM. For preparation, equal amounts of CWS and TDM suspended in chloroform-methanol (95:5) were placed in a glass grinder. The material was dried under nitrogen and then homogenized with 0.2% Tween-PBS into a homogeneous suspension. The final material was heated at 100°C for 15 minutes to ensure sterility. The final concentration of the material was 1 mg/ml. For clinical administration the material was diluted in 100–500 ml of normal saline and infused iv over 30 minutes.

Both preparations were tested for toxicity in mice and guinea pigs and for sterility using trypticase-soy broth and thioglycollate media at 25°C and 37°C prior to clinical use.

Clinical Monitoring and Immunologic Studies

All patients admitted to these studies were removed from all chemotherapy for at least 4 weeks. No patients received any concurrent chemotherapy, hormonal therapy, or glucocorticoids. All patients were evaluated initially with a complete physical examination, chest roentgenogram, liver-spleen scan, cbcs, liver and renal function tests, and other tests to determine the extent of disease. Pulmonary function test and/or diffusion capacity was obtained prior to and following treatment to detect pulmonary fibrosis.

All blood tests were repeated at 2–4-week intervals, depending on the protocol. Chest x-rays, liver-spleen scan, and other tests as indicated were repeated at the end of the treatment program. During the first treatment, patients receiving iv therapy were hospitalized and the vital signs watched for 8 hours to detect acute toxic effects.

Skin tests were performed on all patients with purified protein derivative (PPD), streptokinase/streptodornase, and *Candida* prior to treatment. Skin tests were repeated at 4–8-week intervals. Results are reported as the maximum induration in millimeters at 48 hours. Serum protein electrophoresis and quantitative immunoglobulins were performed prior to study and at 2-week intervals. Peripheral blood T cells were determined as E rosettes using sheep rbcs. Lymphocyte stimulation by phytohemagglutinin (PHA) was determined using a suboptimal and an optimal concentration of PHA. Blastogenesis was determined by tritiated thymidine incorporation in 72-hour cultures using microliter plates. In the patients receiving the combined CWS/TDM, serum lysozyme, and indomethacin-sensitive (monocyte) suppressor cells (23) using a final concentration of 1 mg/ml of indomethacin were also determined.

Data Analysis

The incidence of clinical side effects is reported as the number and percentage of treatments at a given dose level in which toxicity was observed.

Possible changes in the peripheral blood cell counts, renal or liver function tests, and immune parameters were evaluated by plotting each parameter for all patients as a function of the total dose of immunotherapy received. This was done separately for the CWS and the CWS/TDM groups. We then determined if a change occurred with treatment by calculating the least-squares straight-line regression and determining if the slope of the line was significantly different from zero. This method of analysis permits the determination of small but significant changes as a function of the drug dose received. Such small changes may be within the broad range of normal for that particular parameter and not be detected by the usual approach of evaluating only for the appearance of abnormal values. The results are given as the slope of the line and the probability that it represents a significant change.

RESULTS

Patient Population and Treatment Program

A total of 26 patients received either CWS or CWS/TDM. Thirteen patients were given iv suspensions of *M. smegmatis* CWS. Patients were treated every 2 weeks for a total of four treatments over an 8-week period. Each patient received the same dose throughout his or her treatment. The dose varied from 100 to 1000 $\mu g/m^2$. Of these 13 patients, eight had malignant melanoma and five had metastatic hypernephroma. Three patients had received prior chemotherapy. Three patients had received prior hormonal therapy. All patients had a good performance status ($\geq$ 70, Karnofsky) and an expected survival of at least 3 months.

A second, separate group of 13 patients received iv CWS combined with an equal amount of TDM. Of these 13 patients, seven had malignant melanoma, three had

carcinoma of the lung, and one each had hypernephroma, lymphoma, and sweat gland carcinoma. All patients had a good performance status (≥ 70, Karnofsky) and a life expectancy of 3 months. Because the initial group had tolerated the iv CWS well, patients receiving the combined CWS/TDM were treated weekly, instead of every 2 weeks, for up to 8 weeks. The weekly dose varied from 100 to 1000 $\mu g/m^2$ and was constant for each patient. Therapy was discontinued prior to 8 weeks in six of the 13 patients. Therapy was discontinued in three patients because of rapidly progressive disease. One patient at a dose level of 100 $\mu g/m^2$ of CWS/TDM and two patients at a dose level of 500 $\mu g/m^2$ of CWS/TDM refused further therapy because of side effects, mostly chills and fever.

Toxicity of Iv CWS and CWS/TDM

A total of 52 courses of CWS and 84 courses of CWS/TDM were given. The observed clinical side effects were shaking chills and fever. The incidence of chills for the two treatment groups is given in table 1. Most patients had moderate shaking chills, which usually occurred 1-2 hours after the infusion. The most severe chills occurred in the patients receiving 1000 $\mu g/m^2$ of the CWS/TDM. The chills were typically relieved within 15 minutes by giving 25-50 mg of meperidine hydrochloride iv. The incidence of fever following administration of the drugs is shown in table 2. Fever was more common in patients receiving CWS alone (92% of doses) than in patients receiving the combined CWS/TDM (59% of doses).

There were no other clinical side effects. Sequential blood pressure measurements following therapy showed no change.

Effect on Hematologic, Renal, Hepatic, and Pulmonary Parameters

The changes (if any) in various clinical laboratory parameters and their significance are shown in table 3. There was a slight decrease in hemoglobin in the CWS group. Both groups had a significant increase in the wbc count, and there was a significant increase in the platelet count in the CWS/TDM group. There was no consistent effect on renal function. The sequential measurement of hepatic function showed a significant increase in alkaline phosphatase only in those patients receiving CWS/TDM. Pulmonary function, measured in patients prior to and following therapy, showed no significant changes in either group (data not shown). Coagulation studies consisting of partial thromboplastin time, protime, and thrombin time were sequentially measured. No significant changes occurred (data not shown).

Effect on Serum Proteins and Immunoglobulins

The change (if any) in serum proteins and immunoglobulins occurring with therapy is shown in table 4. There were small increases noted in the IgM and Alpha 1 in the group receiving CWS. No significant changes were noted in the group receiving CWS/TDM.

Effect on Immune Parameters

A battery of measurements of cellular immune populations was made in one or both of the treatment groups. The observed changes in these parameters occurring during therapy are summarized in table 5. The data are expressed as the average change/1000 μg of

TABLE 1.—Incidence of chills in patients receiving iv CWS or CWS/TDM

Agent and dose* ($\mu g/m^2$)	No. of treatments	No. (%) with chills		
		None	Moderate	Severe
CWS				
100	16		16 (100)	
250	8		8 (100)	
500	16		16 (100)	
1000	12	3 (25)	9 (75)	
Total	52	3 (6)	49 (94)	
CWS/TDM				
100	20	9 (45)	11 (55)	
250	22	9 (9)	20 (91)	
500	20	6 (20)	13 (65)	1 (5)
1000	22	8 (36)	13 (59)	1 (5)
Total	84	25 (30)	57 (68)	2 (2)

* Dose is given as μg of CWS. Patients receiving CWS/TDM also received an equal amount of TDM.

TABLE 2.—Incidence of fever in patients receiving iv CWS or CWS/TDM

Agent and dose* ($\mu g/m^2$)	No. of treatments	No. (%) with fever		
		None	< 38.5°C	> 38.5°C
CWS				
100	16		16 (100)	
250	8		8 (100)	
500	16		8 (50)	8 (50)
1000	12	4 (33)	4 (33)	4 (33)
Total	52	4 (8)	36 (69)	12 (23)
CWS/TDM				
100	20	12 (60)	5 (25)	3 (15)
250	22	2 (9)	11 (50)	9 (41)
500	20	8 (40)	7 (35)	5 (25)
1000	22	12 (55)	9 (40)	1 (5)
Total	84	34 (40)	32 (38)	18 (22)

*Dose is given as μg of CWS. Patients receiving CWS/TDM also received an equal amount of TDM.

drug received. In both groups there was a small increase in the percent and total number of lymphocytes, which reached significance in the CWS group. This is expected with the significant increase in wbc count seen in both groups. In the CWS but not the CWS/TDM group there was a small and significant decrease in the monocytes. There were no significant changes in the T-cell population or lymphocyte blastogenesis by PHA. In the CWS/TDM group, indomethacin-sensitive suppressor cells and serum lysozyme were measured for an indication of monocyte activation. A significant increase in the serum lysozyme occurred.

Effect on Skin Test

Skin test data from prior to therapy were compared to skin test data obtained following therapy by use of a chi-square analysis. Seven of the 13 patients in the CWS group were skin-test-positive prior to therapy, showing induration of ≥10 mm to PPD or *Candida* antigen. There was an increase in reactivity (induration) to PPD in two patients in the CWS group (P = 0.0165). Patients receiving CWS were also skin tested for reactivity to intradermal CWS. Induration up to 8 mm was observed prior to therapy using 0.1 μg of aqueous suspension of CWS for skin testing. No change was observed to occur because of the treatment. Two patients receiving the highest dose of CWS (1000 $\mu g/m^2$), who were initially skin-test-positive, became skin-test-negative.

Three of the 13 patients in the CWS/TDM group were initially skin-test-positive with induration of ≥ 10 mm to either PPD or *Candida*. One skin-test-positive patient became skin-test-negative following therapy.

Clinical Efficacy

No tumor regression or response was seen in any of the 2t· patients receiving either of the two preparations.

TABLE 3.—Change in clinical laboratory parameters in patients receiving iv CWS or CWS/TDM

Parameter	Units	Change/1000 μg of drug	
		CWS	CWS/TDM
Hemoglobin	g/dl	-0.12*	0.13
Wbc count	cells/mm^3	0.32*	1.02†
Platelet count	cells/mm^3	18.25	2.61*
BUN	mg/dl	-0.10	-0.56
Creatinine	mg/dl	-0.02	0.00
SGOT	mg/dl	1.09	3.62‡
Alkaline phosphatase	mg/dl	8.54	49.90*
LDH	mg/dl	21.70	11.40
Gamma glutamyl tranferase	mg/dl	5.27	5.26

*$P < 0.01$.
†$P < 0.001$.
‡$P < 0.05$.

TABLE 4.—Change in serum proteins and immunoglobulins in patients receiving iv CWS or CWS/TDM

Parameter	Change in mg/dl/1000 μg of drug received	
	CWS	CWS/TDM
IgG	36.93	-0.52
IgM	12.41*	0.37
IgA	3.69	-0.15
Albumin	-0.05	-0.02
Alpha 1	0.04†	0.00
Alpha 2	0.03	0.00
Beta	0.01	0.00
Gamma	0.03	0.00

*$P < 0.05$.
†$P < 0.01$.

DISCUSSION

In the current study both the CWS and CWS/TDM were tolerated. The major side effects were chills and fever. There was no renal injury. In both studies there was evidence of hepatic change. This was, however, much more apparent in the CWS/TDM study. TDM itself induces a strong cellular reaction and pulmonary granuloma formation in mice (24). It is therefore not unexpected that the combined CWS/TDM would cause more apparent hepatic changes. In the CWS/TDM group, there was no change in serum coagulation studies.

No change in pulmonary function or in chest roentgenogram was seen in these patients. A study of the iv use of MER in dogs showed no evidence of pulmonary and liver epithelial granulomas (21). However, a study of iv MER in man described instances of pulmonary infiltrate (20). This was believed to be related to a hypersensitivity reaction to tuberculin protein and probably represents a different lesion than the epithelioid reaction to the TDM observed in mice (24). In our preparations, protein was removed by treatment with proteolytic enzymes, thus largely eliminating the probability of inducing an allergic pneumonitis.

In both groups, a significant increase in the wbc count occurred with therapy. In the CWS group, there was a significant increase in the number of lymphocytes but not in the percent of lymphocytes. This undoubtedly reflects the increase in the wbc count. A small increase in the percent and number of lymphocytes, which was not significant, was also observed in the CWS/TDM group.

In the CWS group, a small but significant decrease in the number of monocytes was observed despite the overall increase in the wbc count. The reason for this is unknown. It may represent peripheralization of activated monocytes. Similar changes were not seen in the CWS/TDM group where an increase in monocytes and lymphocytes, although not significant in itself, occurred and is consistent with the increase in the wbc count. Overall, there were no significant changes in lymphocyte blastogenesis by PHA.

In the CWS/TDM group two tests, ie, indomethacin-sensitive suppressor cells and serum lysozyme, were used to obtain evidence of possible macrophage activation. One test, the serum lysozyme, showed a consistent and significant increase. Since tests were done at each visit prior to therapy, it is possible that an initial increase in the activity of peripheral blood monocytes occurring during the first several days may have been overlooked and might have been measured by the suppressor cell assay if it had been done at different times. The serum lysozyme possibly represents a more prolonged measure of monocyte activity. A similar increase in serum lysozyme activity has been previously described in patients receiving iv MER (20).

Overall, skin test reactivity to PPD increased in the group of patients receiving CWS alone. Despite that, it is of note that two patients receiving high doses of iv CWS became anergic. In the group receiving CWS/TDM, most patients were initially skin-test-nega-

TABLE 5.—Changes in immune parameters in patients receiving iv CWS or CWS/TDM

Parameter	Units	Change/1000 μg of drug	
		CWS	CWS/TDM
% lymphocytes		1.68	2.61
Lymphocyte No.	cells/mm^3	152.77*	27.26
% monocytes		-1.27†	0.00
Monocyte No.	cells/mm^3	-8.19*	19.78
% T cells		1.52	0.00
T cell No.	cells/mm^3	-5.57	-3.10
PHA-suboptimal	cpm	175.00	4169.00
PHA-optimal	cpm	-1644.00	6600.00
Indomethacin suppressor cells	% change		0.01
Lysozyme	mg/dl		1.35‡

*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

tive. One initially positive patient did, however, also become negative following treatment with CWS/TDM. This conversion, especially at high doses, may also reflect the activity of suppressor cell (activated) macrophages. The development of anergy and the laboratory evidence indicate monocyte activations in those patients receiving CWS and suggest that these events are related. It may be possible that in vivo-activated monocytes can suppress the expression of an antigen-specific delayed hypersensitivity reaction.

No antitumor effect was seen. However, laboratory evidence suggests that a state of increased monocyte activity was achieved and therefore some response might be expected. This was a phase I trial and not intended to detect clinical activity. The fact, however, that an in vitro effect can be measured indicated that this may be effective, depending upon parameters of time, frequency, dose, and duration of treatment. Alternatively, the potential deleterious effect of inducing suppressor cells must be recognized. Further studies evaluating these materials and further purification and/or synthesis of the components will hopefully clarify these questions about the potential of these agents. In addition, frequent measurements of monocyte and lymphocyte activity during the first several days following treatment may more readily detect significant changes and a dose-response curve.

We conclude that suspensions of mycobacterial CWS and TDM can be given safely to humans, with evidence of an effect on hematologic parameters and monocyte activity.

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Phase I Study of Continuous Venous Infusion of Floxuridine (5-FUDR) Chemotherapy[1,2]

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Floxuridine (5-FUDR) was administered by continuous venous infusion using an ambulatory portable pump in a phase I study designed to establish the optimal (maximal) daily dose rate to deliver a minimum of 14 days of drug therapy. The major determinant of toxicity was dose rate rather than cumulative dose. Patients receiving > 0.15 mg/kg/day developed dose-limiting diarrhea at 7–14 days (median, 10), while patients receiving 0.1–0.125 mg/kg/day were treated for 18–78 days without adverse effects. The recommended daily dose rate for continuous-infusion schedules of 14 days is 0.15 mg/kg/day. [Cancer Treat Rep 67:791–793, 1983]

The continuous-infusion schedule for delivery of cancer chemotherapy has theoretic advantages based on considerations of drug pharmacology and tumor cell cytokinetics (1). In addition, clinical trials have demonstrated an improved therapeutic index for a number of cytotoxic agents with the continuous-infusion schedule (2–5). 5-FU is an antipyrimidine with an abbreviated half-life (6) in which the continuous-infusion schedule may be superior to bolus schedules on the basis of at least one prospective randomized trial (7), suggesting an increased response rate with a short-term infusion.

5-FUDR is an antipyrimidine which has been previously demonstrated to have potential superiority over 5-FU (8,9). The continuous-infusion schedule for 5-FUDR results in a decrease in the tolerated dose (10) compared to bolus or pulse schedules. We undertook a phase I trial and pilot study to establish the optimally tolerated daily dose rate of continuous venous infusion of 5-FUDR with the goal of inaugurating a phase III comparative trial of systemic venous versus hepatic arterial infusion of 5-FUDR.

METHODS

Eligible patients must have had advanced malignancy without alternative standard forms of therapy. Patients must have had a life expectancy of > 30 days and must have maintained a performance status of 2 or better. Measurable disease parameters were not a prerequisite and patients may have received prior chemotherapy or radiation therapy but could not receive concomitant tumor-specific therapy during the period of drug infusion. Informed consent was a prerequisite for all patient entries. Normal hematologic parameters were essential but abnormalities of renal, pulmonary, or hepatic function did not preclude patient entry. Finally, it was required that prior chemotherapy had been discontinued for ≥ 4 weeks.

Venous access was obtained using the tunneled subclavian line, the technique for which has been described previously (11). The ambulatory infusion pump employed was the Cormed Model ML-6 containing a drug reservoir container of 50 ml. 5-FUDR was reconstituted with normal saline and the dilution was adjusted to provide a minimum daily infusate of 10 ml to optimize pump efficiency.

Patients were monitored weekly and tolerance to treatment was determined by interview for the presence of gastrointestinal effects including nausea, vomiting, or diarrhea and for stomatitis. In addition, weekly cbcs were obtained and sites of measurable tumor were evaluated monthly. The drug infusion was discontinued upon the first identification of toxicity.

A progressive scheme for dose escalation was planned with three patient entries at each dose rate and escalation of 0.025 mg/kg/day between patient groups. The minimum projected duration of treatment at each dose rate level was 14 days without developing adverse effects necessitating discontinuation or interruption of treatment. The starting dose for this study, however, was in

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excess of that tolerated for the projected duration of treatment. Therefore, the actual performance of this phase I study was reversed with de-escalation to the optimal tolerated dose rate.

For patients not demonstrating toxicity, therapy was continued indefinitely. For patients developing toxicity, treatment was interrupted and reinstituted upon recovery at three-quarters of the previous daily dose rate.

RESULTS

Fifteen patients were entered in the study and received a total of 18 courses of therapy. Ten of the 15 patients had metastatic colon cancer, three had gastric cancer, and one each had hepatoma and an unknown primary tumor. Eight of the 15 patients had received prior 5-FU, and none had demonstrated a response to such therapy.

Patients were entered at five dose levels ranging from 0.1 to 0.2 mg/kg/day, although the sequence of entry was from higher to lower dose rates (table 1). Four patient entries at the 0.1-mg/kg/day dose achieved a total cumulative dose of 1.8–7.8 mg/kg, with a cumulative duration of 18–78 days without adverse effects. Two patients received 0.125 mg/kg/day for 30 and 38 days, respectively. Therapy was discontinued in all six patients because of convenience or progressive disease, with no patient experiencing toxic reactions.

The duration of 5-FUDR therapy was limited by the development of stomatitis and diarrhea beginning at a dose rate of 0.15 mg/kg/day. In four patients (five courses) treated at this dose level, diarrhea was the major toxic manifestation, with a cumulative duration of therapy of 9–28 days and a maximum cumulative dose of 1.35–4.2 mg/kg. The median time on study within this group was 16 days. At doses of 0.175 and 0.2 mg/kg/day, the cumulative days on treatment were < 2 weeks, with seven patients developing stomatitis and/or diarrhea. The range of cumulative doses at these two dose rates was 1.05–2.8 mg/kg.

Eleven of 15 patients had measurable lesions and were evaluable for response. There were no instances of objective tumor response by standard clinical parameters, although one patient demonstrated a 30% decrease in the sum of three subcostal measurements of hepatomegaly and one patient demonstrated a 50% decrease in circulating plasma carcinoembryonic antigen.

DISCUSSION

This study confirms previous observations that the tolerated dose of 5-FUDR is decreased when the schedule of delivery is provided by continuous infusion. Studies by Sullivan et al (10) demonstrated that 3 mg/kg/day was the maximum dose one could deliver by continuous infu-

TABLE 1.—Cumulative dose and duration of 5-FUDR delivered by constant venous infusion at graded dose increments with dose-limiting toxicity

Course No.	Dose rate (mg/kg/day)	Total days*	Cumulative dose (mg/kg)	Toxic effects
1	0.1	18	1.8	None
2	0.1	26	2.6	None
3	0.1	34	3.4	None
4	0.1	78	7.8	None
		(30)		
5	0.125	30	3.75	None
6	0.125	38	4.75	None
		(34)		
7	0.15	9	1.35	Diarrhea
8	0.15	15	2.25	Diarrhea
9	0.15	16	2.4	Stomatitis
10	0.15	21	3.1	Diarrhea
11	0.15	28	4.2	Abdominal discomfort
		(16)		
12	0.175	6	1.05	Diarrhea
13	0.175	7	1.25	Diarrhea
14	0.175	11	1.93	Diarrhea
15	0.175	11	1.93	Diarrhea
		(9)		
16	0.2	8	1.6	Diarrhea
17	0.2	10	2.0	Stomatitis/diarrhea
18	0.2	14	2.8	Stomatitis/diarrhea
		(10)		

* Nos. in parentheses = median No. of days for that dose rate.

sion for 5 days, while ten times that dose or 30 mg/kg/day could be delivered for the same duration by bolus injection. The major objective of the present study was to determine the optimally tolerated dose rate which could provide for at least 2 weeks of continuous therapy. Dose-limiting toxicity was manifest as diarrhea with or without stomatitis and the critical determinant of toxicity was the dose rate of delivery. Patients receiving 5-FUDR at doses > 0.15 mg/kg/day experienced dose-limiting diarrhea with or without stomatitis at 2 weeks of continuous treatment. At dose rates of < 0.15 mg/kg/day, most patients could be treated for indefinite intervals for > 30 days without toxicity.

Clinical trials employing 5-FUDR by the continuous-infusion schedule have predominantly involved regional arterial infusion. In such studies, 0.3–0.5 mg/kg/day is delivered initially for an interval of 2–4 weeks but this dose rate is limited by topical toxicity, ie, gastritis and chemical hepatitis. These regional effects restrict the dose rate of the arterial infusion in subsequent courses to doses of 0.1–0.175 mg/kg/day, particularly for the implanted pumps. The recommended dose rate delivered by venous infusion in the present study is 0.15 mg/kg/day, which is comparable to the arterial-infusion dose schedule.

Prospective phase III trials to compare continuous venous infusion with hepatic arterial infusion of 5-FUDR should employ a dose of 0.125–0.15 mg/kg/day if the 2 weeks on and 2 weeks off schedule is planned. For longer durations of continuous venous infusion, the optimal dose rate is 0.125 mg/kg/day.

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Phase I Clinical Trial of *cis*-Dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) (CHIP)[1,2]

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cis-Dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) (CHIP), a second-generation platinum complex with little or no nephrotoxicity in preclinical studies, has undergone phase I clinical testing and initial pharmacokinetic evaluation in 26 patients with solid tumors who received 40 courses of treatment. Doses of 20–350 mg/m² were evaluated as single 2-hr infusions given every 3 weeks for two doses, with 2 L of pretreatment hydration (but no diuretics) in all but five patients. Nausea and vomiting was almost universal but was severe in only three courses. The dose-limiting toxic effect was myelosuppression, with a median wbc count nadir of 2500/mm³ and a median platelet count nadir of 32,000/mm³ at the maximum tolerated dose of 350 mg/m². No increase in serum creatinine or decrease in creatinine clearance was seen. No pretreatment hydration was given in five of the seven patients treated at 350 mg/m². Two cases of hypersensitivity were seen. No hearing loss was observed and no other toxic effects were noted. Plasma decay of total platinum was biexponential with a prolonged β phase. Recovery of platinum in the urine was rapid up to 8–10 hrs and slow thereafter. Recoveries were incomplete and variable (16.5%–62% of the dose at 48 hrs). [Cancer Treat Rep 67:795–800, 1983]

Cisplatin has shown considerable activity clinically against a number of tumors and has now become a part of the standard regimen against nonseminomatous testicular tumors. Its major disadvantage is the renal impairment which can limit therapy with the drug and is often at least partially irreversible (1).

The renal toxicity of cisplatin has led to the search for second-generation platinum compounds which might be free of this toxic effect. One such potential compound, *cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) (CHIP) (fig 1), was initially developed by Cleare et al (2) and evaluated by Connors et al (3). It showed a therapeutic index of 12 (3) against plasmacytoma PC6, and has also been shown to be active against L1210 leukemia, showing an increase in lifespan of 71% after a single administration (4). Against B16 melanoma it showed a maximum T/C of 145%, and against Lewis lung carcinoma it showed a maximum T/C of 188%, with seven cures in ten animals (4).

A comparison of its pharmacokinetics with that of cisplatin in the rat indicated a terminal-phase half-life of total platinum of 14 hrs for CHIP and 54 hrs for cisplatin (5). Studies in the dog showed a terminal-phase half-life of 39.4 hrs (6), which is considerably shorter than that reported for cisplatin (120 hrs) (7).

In toxicology studies, myelosuppression was a major dose-limiting toxic effect of CHIP, and nephrotoxicity was minimal or absent (8). Thus, it seemed to be an important compound to evaluate clinically. An initial clinical trial was carried out to evaluate the dose-limiting toxic effects of this agent in patients with solid tumors.

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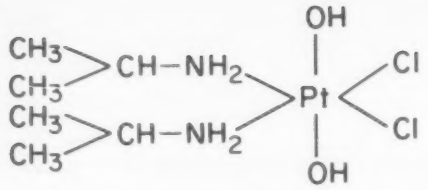

FIGURE 1.—Structure of CHIP.

MATERIALS AND METHODS

CHIP for clinical use was supplied by Bristol Laboratories in 20-mg vials. The drug was dissolved in 250 ml of isotonic saline and was administered over a period of 2 hrs. Administration of the drug was preceded by overnight hydration with 2 L of 5% dextrose in 0.5 N saline, except in five of the seven patients treated at the highest dose (*see* below) in whom pretreatment hydration was omitted.

All patients had advanced cancer refractory to known methods of therapy and had a life expectancy of at least 2 mos. All patients gave written informed consent prior to entry in this study. Patients were required to have a minimum wbc count $> 4000/mm^3$ and a platelet count $> 100,000/mm^3$. Serum bilirubin < 1.5 mg/100 ml, SGOT < 100 IU/ml, and serum creatinine < 1.5 mg/100 ml were also required. The minimum creatinine clearance for entry in the study was 70 ml/min.

Before treatment and following each course of treatment, cbc and platelet count were done twice weekly, and serum Na^+, K^+, Ca^{++}, and PO_4^{---}; creatinine; uric acid; total protein; albumin; bilirubin; alkaline phosphatase; LDH; SGOT; and BUN were done weekly. The 24-hr creatinine clearance was measured before each drug dose and 3 weeks after the final dose. Audiometry at frequencies of 250–8000 cycles/sec (Tracor RA 115A, Rudmose Clinical Audiometer) was performed before entry in the study and 4 weeks after the second dose. Patients who received more than two doses had audiometry before alternate doses and 4 weeks after completion of treatment with CHIP.

Except as noted below, each patient received two doses of the drug. In the absence of toxicity, the second dose was given on Day 22. When toxicity was observed with the first dose, the second dose was given when the wbc count was $> 4000/mm^3$ and the platelet count was $> 100,000/mm^3$.

A course consisted of two doses. Patients who were considered to have benefited from the drug continued treatment as long as benefit was maintained, with adjustment of dose to patient tolerance. The dose was not escalated to toxicity in the same patient. However, after a suitable interval (a minimum of 8 weeks) to exclude the possibility of cumulative toxicity, patients who had received a low dose and had developed no toxic effects, except nausea and vomiting, were permitted to be reentered in the study at a higher dose level.

The drug dose was escalated by 100% increments until initial signs of toxicity, other than nausea and vomiting, were noted. Thereafter, dose escalation was by 50% to moderate reversible toxicity.

In patients undergoing pharmacokinetic studies, blood (5–10 ml) was drawn at midinfusion, at the end of infusion and at 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 hrs, and in selected cases at 36, 48, and 72 hrs, after the end of infusion. Urine was collected at the end of infusion, at 2, 4, 6, and 8 hrs after infusion, and as it was voided thereafter.

Blood was centrifuged immediately and plasma and urine were analyzed for platinum by flameless atomic absorption spectroscopy using either a Perkin Elmer Model 403 atomic absorption spectrophotometer equipped with a 2100 graphite furnace or an IL 457 atomic absorption spectrophotometer (Instrumentation Laboratories) equipped with a 655 atomizer and a fastac 254 autosampler. The conditions for using the Perkin Elmer spectrophotometer were as described previously (6). For the IL 457, a five-stage temperature program, with atomization for 4 secs at 2400°C, was used. Argon was the purge gas. Plasma samples were digested with an equal volume of 70% HNO_3 and the residue was redissolved in 0.5% HCl; 20–50-μl volumes of this solution were injected into the furnace. The detection limit under these conditions was 35 ng/ml. The two instruments gave equivalent results.

RESULTS

A total of 26 patients (19 males and seven females) were entered in the study and received 40 courses of treatment; the median age was 60 years (range, 23–72). Tumors were diagnosed as follows: carcinoma of the large bowel (16 patients), transitional cell carcinoma of the bladder (two), squamous cell carcinoma of the lung (one), renal cell carcinoma (one), adenocarcinoma of the breast and pancreas (one each), sarcoma of the prostate, pancreas, and soft tissue (one each), and melanoma (one). All 26 patients had had prior chemotherapy, 25 had had prior surgery, and five, in addition, had had prior radiotherapy.

Nausea and vomiting, defined as mild (nausea and one to two episodes of vomiting), moderate (nausea and vomiting lasting up to 4 hrs, easily controlled by antiemetics and not requiring parenteral fluids), or severe (vomiting lasting > 4 hrs and occasionally requiring parenteral support), was seen at all doses > 20 mg/m^2. It was mild in 10%, moderate in 80%, and severe in 10% of the courses. The dose-limiting toxic effect was myelosuppression. This was seen sporadically in heavily pretreated patients at doses up to 120 mg/m^2 but was inconsequential. Toxicity of the three highest doses (180, 270, and 350 mg/m^2)

TABLE 1.—Hematologic toxicity of CHIP (180–350 mg/m²)

	Dose (mg/m²)		
	180	270	350
No. of patients*	5	9	7†
With hematologic toxicity‡	3	8	6
With leukopenia	2	3	6
With thrombocytopenia	1	7	6
Wbc count nadir (cells/mm³)			
Median§	3250	2800	2500
Range	3200–3300	2600–3700	2300–3200
Median days to wbc count nadir	19	21	25
Platelet count nadir (cells/mm³)			
Median§	82,000	53,000	32,000
Range	—	18,000–95,000	8000–74,000
Median days to platelet count nadir	15	15	15

*Some patients receiving > 2 doses because of stable disease were treated with a lower dose after receiving a higher dose. The toxicity of the highest dose that these patients received is that reported in the table.

†Includes 1 patient who received only 1 dose and did not have toxic effects.

‡Either leukopenia (wbcs < 4000/mm³) or thrombocytopenia (platelets < 100,000/mm³).

§Medians for wbc and platelet count nadirs have been calculated from the nadirs of those patients who developed leukopenia or thrombocytopenia, respectively.

is shown in table 1. The maximum tolerated dose was 350 mg/m², with both leukopenia and thrombocytopenia being noted. There was no consistent fall in hematocrit.

No trend was seen in changes in creatinine clearance or serum creatinine either in patients receiving two doses or in seven others who received multiple doses. The data for pre- and post-treatment creatinine clearance and serum creatinine for the highest dose used (350 mg/m²) are shown in table 2. Five of the seven patients (Nos. 3–7) receiving this dose received no pretreatment hydration. It is also important to note that two patients at this dose have received five doses of CHIP, with no change in renal function.

Two patients showed evidence of hypersensitivity reactions to CHIP. One patient, a 55-year-old female with carcinoma of the colon, had received previous treatment with 5-FU but no prior treatment with a platinum complex. Shortly after the infusion of a dose of 270 mg/m², she experienced chills, shortness of breath, and wheezing. Morphine and methylprednisolone were given iv, and diphenhydramine was given orally. The symptoms subsided within 1 hr. The second patient, a 61-year-old male with renal cell carcinoma and only one kidney, had had prior chemotherapy with bleomycin, vincristine, methotrexate, leucovorin, and 5-FU. He received CHIP (270 mg/m²) as an initial dose, with hematologic toxicity but no evidence of hypersensitivity. Six weeks later he received another dose of CHIP (180 mg/m²). At the end of the infusion he experienced shortness of breath, wheezes, pyrexia to 39.3°C, and hypotension. Methylprednisolone, aminophylline, dopamine, and iv fluids were given. The patient's condition stabilized and he was discharged 3 days later, with no sequelae.

No evidence for eighth nerve damage was found in 15 patients in whom follow-up audiograms were obtained.

In patients receiving multiple doses at myelosuppressive dose levels, it was not possible to give the drug every 3 weeks; a minimum interval of 4 weeks between doses

TABLE 2.—Renal function after CHIP (350 mg/m²)

Patient No.	Serum creatinine (mg/dl)		Creatinine clearance (ml/min)	
	Pretreatment	Post-treatment*	Pretreatment	Post-treatment*
1	0.7	0.6	100	117
2	0.8	0.8	142	155
3	0.6	0.6	—	—
4	1.1	1.0	105	113
5	0.9	0.8	110	110
6	0.8	0.7	120	154
7	1.3	1.3	—	—

*At the time of the 2nd dose.

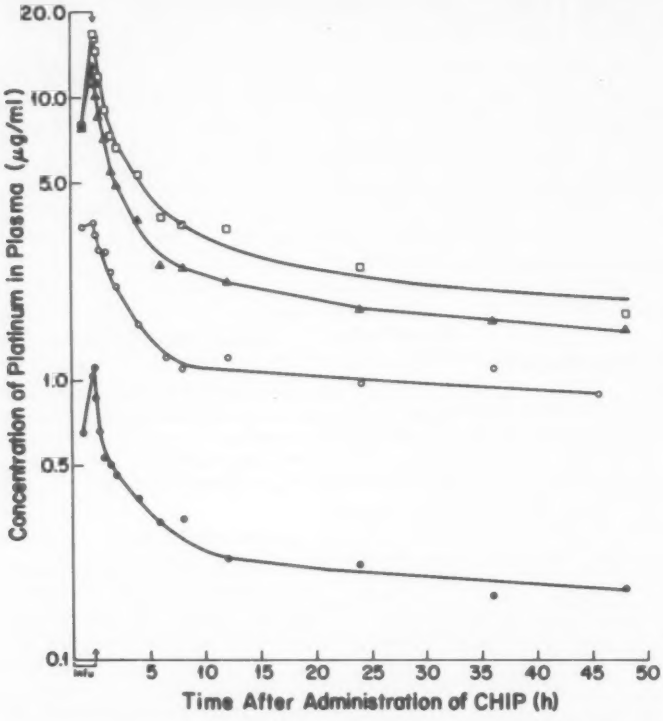

FIGURE 2.—Mean plasma decay of total platinum following infusion of CHIP at doses of 20 mg/m^2 (●, N = 3), 120 mg/m^2 (○, N = 3), 270 mg/m^2 (▲, N = 3), and 350 mg/m^2 (□, N = 4). Symbols represent mean values for the raw data. Curves were drawn by visual estimation of the best fit.

was required for recovery of the bone marrow, particularly the platelet count.

No partial responses were seen. Five patients received more than two doses because of apparently stable disease lasting 2–10 months.

In pharmacokinetic studies plasma platinum decayed biexponentially following the end of the infusion. Figure 2 shows the plasma decay curves for those doses for which data are available for three or more patients. It can be seen that the β phase of the plasma decay is prolonged. Urinary excretion of platinum after CHIP, as shown in figure 3, is initially rapid but after 8–10 hrs little excretion occurs. Recovery is incomplete and variable, being in the range of 16.5%–63% of the dose for 48 hrs (fig 3).

DISCUSSION

CHIP was introduced into clinical trial because in preclinical tumor systems it showed a level of antitumor activity which was either equal to or in excess of that of cisplatin (3,4). In preclinical toxicology, the drug showed either no or minimal renal toxicity at toxic dose levels (7). In addition, the drug is of interest in being the only complex of quadrivalent platinum to be introduced so far into the clinic. Platinum complexes which have been tested so far have been complexes of divalent platinum which are square planar complexes. Complexes of quadrivalent platinum have an octahedral configuration. It is possible, therefore, that such compounds might show interesting differences in antitumor spectrum from that of cisplatin.

The anticipated change in spectrum of toxicity predicted from rat and dog toxicity data was found with CHIP clinically. The dose-limiting toxic effect was myelosuppression in all cases, thrombocytopenia being somewhat more severe than leukopenia; even in patients in whom no pretreatment hydration was given, renal toxicity was not seen. This makes it likely that CHIP will be useful in patients with tumors sensitive to cisplatin who are unable to receive this drug because of renal impairment.

The occurrence of hypersensitivity reactions is a feature of treatment with cisplatin and was also found with CHIP. Because of the small numbers it is not possible to predict whether this will be more or less common as a side effect of CHIP than of cisplatin. It is of interest that

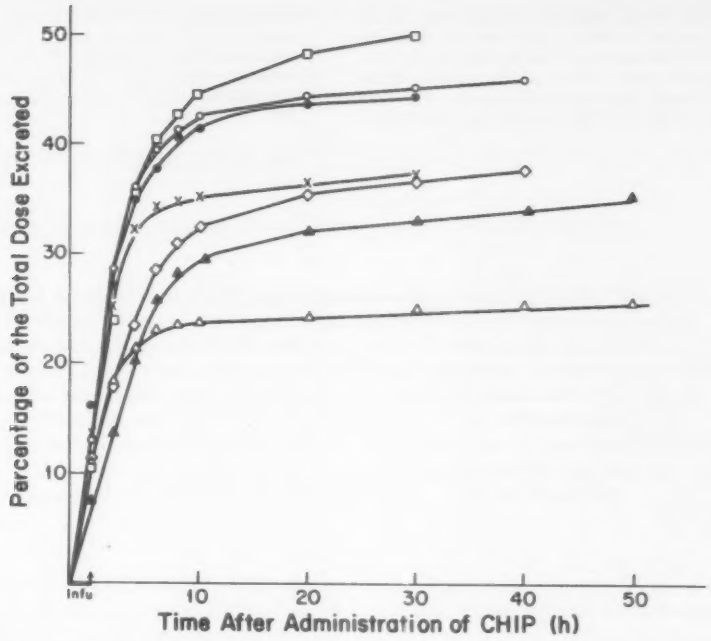

FIGURE 3.—Mean urinary excretion of total platinum after infusion of CHIP at doses of 20 mg/m^2 (●, N = 2), 40 mg/m^2 (X, N = 3), 80 mg/m^2 (Δ, N = 2), 120 mg/m^2 (○, N = 4), 180 mg/m^2 (□, N = 3), 270 mg/m^2 (▲, N = 3), and 350 mg/m^2 (□, N = 3). Symbols represent mean values for the raw data. Curves were drawn by visual estimation of the best fit.

a patient with a nonseminomatous testicular tumor (not included here because he was treated with CHIP and bleomycin, having progressed on cisplatin and bleomycin) had no reaction to CHIP although he had a hypersensitivity reaction to cisplatin requiring steroids before each dose.

Unlike the situation in our animal studies in which platinum kinetics after CHIP showed a somewhat more rapid excretion and a considerably shorter β-half-life than after cisplatin (5,6), the kinetics in man looks remarkably similar to that reported for cisplatin. From these preliminary data it does not appear that the difference in the amount of drug tolerated (approximately three times more of CHIP than cisplatin) or spectrum of toxicity can be explained on the basis of differences in pharmacokinetics of platinum metal. However, it is known that for cisplatin in man and for CHIP in dogs, pharmacokinetics of the drug differs markedly from that of total platinum; the unchanged drug in each case has a half-life of < 1 hr, whereas the total plasma platinum, because of extensive protein binding, has a half-life of several days. These data cannot, therefore, be used as an accurate guide to the fate of the unchanged drug (6,9). The data for the pharmacokinetics of non-protein-bound platinum and unchanged CHIP are under development and will form the subject of a separate communication.

Because of the sometimes severe thrombocytopenia seen after a dose of 350 mg/m^2, this dose is not recommended as a starting dose for phase II studies, which should start at a dose of 270 mg/m^2 in patients with good marrow reserve and at 180 mg/m^2 in patients with extensive prior therapy and poor marrow reserve. The drug should be given every 4–6 weeks. The necessity for the 2 L of prehydration and the 2-hr drug infusion used in the present study is under evaluation.

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Phase I Trial of Homoharringtonine[1,2,3]

James A. Neidhart,* Donn C. Young, Diane Derocher, and Earl N. Metz[4,5]

Homoharringtonine is one of a group of cephalotaxine esters reported by the Chinese to have significant antitumor efficacy, with particularly good activity in human leukemias. Preclinical antitumor activity against P388 leukemia, colon 38 carcinoma, and mammary carcinoma led to phase I trials which are currently nearing completion in this country. The phase I study reported here used a single infusion of homoharringtonine over 90 minutes given every 21 days. Dose-related and life-threatening hypotension, without significant myelosuppression, was the dose-limiting toxic effect. The mechanism of hypotension is not understood but it is not related to cardiac arrhythmias. Myelosuppression was not seen regularly. We do not recommend this schedule for phase II trials. [Cancer Treat Rep 67:801–804, 1983]

Homoharringtonine is one of a group of cephalotaxine esters originally isolated from the wood and bark of an evergreen, *Cephalotaxus harringtonia*, found on the Chinese mainland (1). Cephalotaxine esters had long been used as antitumor agents in folk medicine. In 1976 the Chinese reported antileukemic activity using a mixture of homoharringtonine and harringtonine (2). Preclinical testing in this country showed good activity against intraperitoneal P388 leukemia when homoharringtonine was given ip, sc, iv, or orally. Activity in solid tumors was seen against colon 38 tumors and $CD8F_1$ mammary carcinoma, with the drug producing cures in both systems. Minimal activity was seen against B16 melanoma, and the drug was inactive against Lewis lung tumors and the three human tumor xenografts tested. Activity is highly schedule-dependent, with the greatest effect seen using once daily dosing for 9 days (3). Homoharringtonine inhibits protein synthesis with secondary inhibition of DNA and RNA synthesis (4). Inhibition of protein synthesis is reversible at low drug concentrations in vitro. Toxic effects in dogs included myelosuppression, gastrointestinal upset, hepatic dysfunction, and cardiac arrhythmias.

This study was designed to determine the optimal dose and dose-limiting toxic effects of homoharringtonine given as a single infusion every 21 days.

STUDY DESIGN

Patient characteristics for the 30 patients entered are shown in table 1. All patients were adults, and performance was near normal in 16. Pretreatment was minimal: nine patients had had no prior therapy, and only three had had two or more prior chemotherapeutic regimens. Patients with a Zubrod performance status of 3 were entered in the study if their life expectancy was judged to be two full courses of therapy. The two patients with a performance status of 4 had clinical deterioration between electing treatment with homoharringtonine and receiving the drug, but they decided to proceed. Patients with a clinical history of congestive heart failure or cardiac arrhythmias requiring treatment were excluded. One patient with erythroleukemia and one with marrow involvement by lymphoma were not evaluable for hematologic toxicity. Twenty-seven patients were evaluable for hematologic toxicity. All patients gave written and oral informed consent to receive homoharringtonine.

Dosing was started at $\frac{1}{10}$ the murine LD10 (1.3 mg/m^2).

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[2]Supported by contract N01-CM-27509 (Division of Cancer Treatment) and Public Health Service grant CA-16058 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Homoharringtonine was supplied by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[4]Division of Hematology and Oncology, Department of Medicine (*J. A. Neidhart* and *E. N. Metz*), and the Comprehensive Cancer Center (*J. A. Neidhart*, *D. C. Young*, *D. Derocher*, and *E. N. Metz*), The Ohio State University, Columbus.

[5]We thank the nurses of the Interdisciplinary Oncology Unit for the concerned and alert care of their patients which led to early definition of hypotension as a toxic effect, and Joan Loomis for her assistance in manuscript preparation.

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	30
Evaluable for hematologic toxicity	27
Evaluable for nonhematologic toxicity	29
Median age in yrs (range): 57 (24-81)	
Performance status (Zubrod)	
0-1	16 (53%)
2-3	11 (36%)
4	2 (6.7%)
Sex	
Male	21
Female	9
Prior therapy	
None	9 (30%)
Chemotherapy only	11 (37%)
Chemotherapy and radiation therapy	10 (33%)
Diagnosis	
Colon cancer	6
Lung cancer	3
Lymphoma	4
Breast cancer	3
Pancreatic cancer	3
Esophageal cancer	3
Sarcoma	2
Prostatic cancer	2
Erythroleukemia, bladder cancer, melanoma, renal cell carcinoma	1 each

Doses were escalated using a modified Fibonacci scheme, with dose levels as shown in table 2. Dose escalations were permitted in individual patients after three courses if no toxicity was noted. Homoharringtonine was given as a single iv infusion over 10 minutes initially, but over 90 minutes in doses > 6.4 mg/m^2. Courses were repeated every 21 days.

Cbcs were obtained weekly throughout the study. Liver enzymes (SGOT, SGPT, and alkaline phosphatase) were obtained weekly after the first course of therapy and then prior to each subsequent course. Serum electrolytes, BUN, creatinine, glucose, urinalysis, prothrombin and partial thromboplastin times, and an ECG were obtained prior to each course of therapy for the first three courses. A noninvasive study of cardiac function (systolic time intervals or MUGA scan) was obtained prior to treatment. For all patients except two, a Holder monitor was used to follow cardiac rhythm for 24 hours following the first dose. At least the initial three courses were given on the Interdisciplinary Oncology Unit of the Comprehensive Cancer Center. Vital signs were monitored every 2 hours after drug administration for approximately 24 hours once hypotension was identified as a potential toxic effect.

Measurable tumor was not a requirement for patient entry but, if present, responses were recorded prior to each treatment.

RESULTS

Table 2 shows the hematologic data for the 60 evaluable courses of therapy in the 27 patients evaluable for hematologic toxicity. No consistent hematologic toxicity was seen. Mild and reversible leukopenia was seen in three patients. Only two patients developed significant thrombocytopenia. In one case, thrombocytopenia was probably related to sepsis from aspiration pneumonia. The thrombocytopenia in the second patient reversed and was unexplained.

Table 3 shows data regarding hypotension, which was the dose-limiting toxic effect and probably contributed to the death of one patient receiving a 11.5-mg/m^2 dose. The data are presented by dose and by functional capacity of the patient. Patients treated at two dose levels are included at both doses. The lowest systolic and diastolic pressures and the duration of hypotension are also presented, as well as whether hypotension reversed and the total number of courses each patient received. No patient was noted to have hypotension at a dose ≤ 4.2 mg/m^2. Blood pressure was measured every 4 hours at these doses, and mild or transient hypotension might not have been detected. There is a suggestion of a relationship of hypotension to both dose and performance status at higher dose levels. The five patients who did not develop hypotension but received 6.4-9.0 mg/m^2 had a perform-

TABLE 2.—Hematopoietic toxicity

Dose (mg/m^2)	No. of patients entered	No. of evaluable patients/No. of courses	Wbc count (× 10^3 cells/mm^3)			Platelet count (× 10^3 cells/mm^3)		
			Nadir range	Median nadir (Day)	No. of patients suppressed	Nadir range	Median nadir (Day)	No. of patients suppressed
1.3	3	2/5	3.1-8.0	6.1 (17)	0	170.0-253.0	226.5 (8)	0
2.6	4	3/12	3.5-6.7	4.1 (8)	0	99.0-275.0	168.0 (14)	1
4.2	3	3/7	6.4-8.1	7.6 (13)	0	208.0-558.0	329.0 (15)	0
6.4	5	5/11	2.6-9.3	4.2 (10)	1	10.0-413.0	178.5 (16)	1
7.7	7	7/12	2.0-3.0	3.7 (12)	1	29.0-520.0	193.0 (15)	1
9.0	7	5/13	2.3-4.8	4.4 (13.5)	1	150.0-295.0	188.0 (14)	0
11.5	1	0	—		—	—	—	—

TABLE 3.—Hypotension incidence and severity

Dose (mg/m^2)	No. of patients	Functional capacity: Hypotension absent	Functional capacity: Hypotension present	Hypotension: Low value (systolic/diastolic)	Hypotension: Duration (hrs)	Reversibility*
1.3–4.2	8	0,0,0,1,1, 2,3,3				
6.4	6	0,1	1	75/60	3	R(3+)
			3	78/50	6	R(2)
			3	94/70	—	R(1)
			4	90/60	—	R(1)
7.7	7	0,0	0	70/42	3.0	R(2)
			1	60/40	2	R(2)
			2	70/38	4	R(1)
			3	70/50	5	R(2)
			4	68/58	14	I(D)
9.0	10	0	0	80/50	4	R(1)
			0	82/0	4	R(2)
			0	72/50	3.0	R(3)
			1	62/40	2	R(1)
			1	68/50	3	R(2)
			1	62/46	2	R(2)
			1	68/40	5	R(3)
			2	62/44	16	R(3)
			2	72/48	4	R(1)
11.5	1		3	80/40	—	I(D)

*R = reversible; I = irreversible; D = died (values in parentheses = No. of total courses the patient eventually received).

ance status of 0 or 1. All patients who had a performance status of 0 or 1 tolerated hypotension well or proceeded to receive multiple courses. All patients with a performance status of > 1 developed hypotension and tolerated it less well. The patient receiving the 11.5-mg/m^2 dose probably died as a result of the hypotension. This patient was restricted to bed prior to treatment due to pulmonary function compromised by metastatic tumor. Treatment was given at his and his family's insistence. A second patient died before recovery from hypotension, but also had developed aspiration pneumonia secondary to esophageal carcinoma. The pneumonia was probably the major and certainly a sufficient cause of death. Hypotension was unresponsive to volume replacement or vasopressors in all patients.

Hypotension was not related to cardiac arrhythmias. Cardiac arrhythmias included asymptomatic short runs of ventricular tachycardia in two patients and a modest increase in ventricular premature contractions in four. Following treatment one patient had a marked increase in premature ventricular contractions to about 35/minute. All patients developed a mild and asymptomatic sinus tachycardia. In no case was the arrhythmia temporally associated with the onset of hypotension, and the two patients who died with hypotension did not show drug-induced rhythm changes other than sinus tachycardia.

Other nonhematologic toxic effects are presented in table 4. Diarrhea was frequently marked, watery, and explosive at the higher doses. Vomiting was frequent and often severe. Chills and fever were seen occasionally. Hematochezia was not seen.

Response

One patient with prostatic carcinoma had a > 50% reduction in one lymph node and a 30% reduction in another lasting 4 months, with no change in bone disease. No other responses were seen.

DISCUSSION

Homoharringtonine is an agent of considerable interest mainly because of the antileukemic effect reported from the Chinese mainland. The antitumor activity, however, may be dose- and schedule-related. Preclinical efficacy studies in this country found a frequent dosing regimen to be most effective. The Chinese have most frequently used a repeated daily dosing scheme for 7–14 days and have not reported use of an intermittent high-dose regimen. The dosing scheme used in this study is one of the three typically used in phase I studies. Hypotension is clearly dose-limiting and probably is a prohibitive toxic effect for many patients above a dose of 6.4 mg/m^2. Slowing the infusion rate to 90 minutes and volume expansion with therapy did not prevent or ameliorate hypotension. The mechanism of hypotension is not clear but it is not related to cardiac rhythm changes. Patients did not appear to have peripheral vasodilation, and vasopressors were ineffective in treating this hypotension.

TABLE 4.—Nonhematologic toxicity

Dose (mg/m^2)	No. of patients/No. of courses	Vomiting, grade*—†			Diarrhea†	Other†
		1	2	3		
1.3	3/7	0	0	0	0	0
2.6	4/12	2	3	0	0	0
4.2	3/7	0	2	1	0	0
6.4	5/11	0	3	3	1	Dizziness and chills (2)
7.7	7/15	0	3	6	1	Alopecia (3) and anorexia (1)
9.0	9/13	1	3	4	1	Chills (2)
11.5	1/1	0	1	0	1	

*Grade 1 = nausea; 2 = vomiting controlled by antiemetics; and 3 = vomiting not controlled by antiemetics.
†Values = No. of courses

We do not recommend that this schedule be used in phase II studies. Other schedules are currently being explored. Daily administration may be safe if single doses are kept to < 4 mg/m^2 or if the daily dose is divided. Continuous-infusion regimens over 24 hours or several days are being explored. Cooney et al (5) reported the use of continuous infusion over 5 days and had not reached dose-limiting toxicity at 3.75 mg/m^2/day. Qualitative toxicity seems to be highly schedule-dependent, since they saw primarily myelosuppression, with no hypotension or cardiac arrhythmias. Regardless of schedule, all patients should have blood pressure monitored carefully until the incidence and severity of hypotension is defined for each schedule.

Homoharringtonine remains a potentially useful and interesting new agent, and phase II studies will start soon. Hopefully, a frequent or continuous dosing schedule will be the least toxic and also the most effective.

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Pharmacokinetics of Mitomycin C in Patients Receiving the Drug Alone or in Combination[1,2,3]

Guy A. van Hazel, Mark Scott, Joseph Rubin, Charles G. Moertel, Robert T. Eagan, Michael J. O'Connell, and John S. Kovach[4,*]

We have characterized plasma pharmacokinetics in 30 patients receiving mitomycin C (mitomycin) in doses ranging from 6 to 20 mg/m² by iv bolus injection, either alone or in combination with other chemotherapeutic drugs. Plasma elimination of the drug was described by a two-compartment model in all but three cases, giving mean values for α-half-life of 8.2 mins, β-half-life of 51.8 mins, and total body clearance of 332.9 ml/min/m². The mean urinary excretion was 8.1% of the total dose administered. We did not detect alterations in pharmacologic parameters related to liver dysfunction, dose of mitomycin, and concomitant administration of other cytotoxic drugs. [Cancer Treat Rep 67:805–810, 1983]

After having disappointing early trials in this country, mitomycin C (mitomycin) has now been established as a chemotherapeutic agent with a broad spectrum of activity, and it is increasingly being incorporated into combination chemotherapy (1). Early studies are attempting to define its role in high-dose therapy followed by autologous marrow (2) and hepatic artery infusions both as a free drug (3) and in slow-release formulations (4). With this renewed interest in the drug, new attention has been focused on its pharmacokinetics.

Using a microbiologic assay, Fujita (5) reported dose-dependent pharmacokinetic behavior of mitomycin in patients receiving a total of 2, 10, 20, and 30 mg. In 1979, Kono et al (6) published a high-pressure liquid chromatographic (HPLC) assay and data on plasma elimination in a single patient. Using an HPLC assay similar to Kono, den Hartigh et al (7,8) reported a pharmacokinetic study of mitomycin in 26 patients. They noted large variations in kinetics of mitomycin and suggested that saturation of an elimination pathway occurred at high-dose levels. Using another modification (9) of the HPLC assay of Kono, we have characterized the plasma pharmacokinetics and urinary excretion in 30 patients receiving mitomycin alone or in combination with other chemotherapeutic agents.

MATERIALS AND METHODS

Drug Administration and Patient Samples

Mitomycin was purchased from Bristol Laboratories (Syracuse, NY). Patients receiving mitomycin by rapid iv injection had venous blood samples taken prior to administration of drug and at intervals over 3 hrs from an indwelling catheter kept open with 5% dextrose in water. Whole blood was collected into heparinized (143 units) venoject tubes and centrifuged at 3000 rpm for 5 mins. Rbcs and plasma were separated, and the plasma was frozen at -70°C until analysis. Urine was collected prior to and, at times, up to 24 hrs after mitomycin administration.

Mitomycin Assay

After initial purification of plasma samples by column chromatography using a nonionic resin (Porapak Q), mitomycin and an internal standard (porfiromycin) were measured in plasma and urine by HPLC assay using a Hewlett Packard C8 10μ reversed phase column. A gradient elution changing from 5% to 41% acetonitrile in water over 8 mins followed by a 12-min wash and re-equilibration was employed. Mitomycin and porfiromycin were

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measured by UV absorbance at 360 nm. Details regarding extraction efficiency sensitivity, specificity, and linearity of the assay were previously reported (9).

Data Analysis

Pharmacokinetic parameters were calculated with the NONLIN pharmacokinetic modeling program (10) on a CDC Cyber 170-720 computer. Mitomycin concentration versus time data were fitted to a multiexponential equation ($C = Ae^{-\alpha t} + Be^{-\beta t}$) or to a single exponential equation ($C = Be^{-\beta t}$) where C is mitomycin plasma concentration, t is time after injection, A and B are the intercepts of the components of the curve with the ordinate, and α and β are apparent first-order disposition rate constants. Area under the curve (concentration times time [C × T]) values were calculated from the equation $AUC = A/\alpha + B/\beta$. Weighting factors of the reciprocal of the mitomycin concentration, the square of the reciprocal of the mitomycin concentration, or no factor were used, depending on which gave the best fit of the data.

Differences among means were tested using one-way analysis of variance methodology. The correlation of β-half-life and clearance with renal and liver functions was assessed by using a multiple linear regression program (Fit Multiple) of the PROPHET system (Bolt, Beranek and Newman, Inc, Cambridge, MA). The mean ± SEM for individual concentration time points of patients grouped according to the dose of mitomycin received were calculated and graphed with the aid of the Public Statistics program of the PROPHET system. Two-compartment models were calculated for these mean values by the NONLIN program (10) using a weighting factor of the square of the reciprocal of the concentrations.

RESULTS

Thirty patients received mitomycin as a bolus iv injection. There were 19 males and 11 females. The median age was 61 yrs (range, 43-75). The mean weight ± SEM was 70.3 ± 2.3 kg. Two patients had serum concentrations of creatinine greater than normal (1.4 and 3.1 mg/dl). Seven patients had serum concentrations of direct bilirubin greater than normal, ranging from 0.3 to 3.3 mg/dl. Fifteen patients had serum concentrations of alkaline phosphatase greater than normal, ranging from 206 to 1668 IU/L, and 14 patients had serum concentrations of aspartate transaminase greater than normal, ranging from 32 to 242 IU/L. There were 12 patients treated with mitomycin alone and 18 patients treated with mitomycin in combination with other agents. Of the 18 patients treated with other drugs in addition to mitomycin, one received 30 mg/m^2 of doxorubicin, one received 30 mg/m^2 of doxorubicin and 60 mg/m^2 of platinum, three received 40 mg/m^2 of doxorubicin and 60 mg/m^2 of platinum, two received 30 mg/m^2 of doxorubicin and 600 mg/m^2 of 5-FU, six received 40 mg/m^2 of lomustine and 10 mg/m^2 of methotrexate, and five received 175 mg/m^2 of Baker's antifol. Sixteen patients had had prior chemotherapy. Ten patients had doxorubicin-containing regimens, one of whom was previously exposed to mitomycin and doxorubicin. Table 1 shows the derived pharmacokinetic parameters for each patient. There was wide variation of pharmacokinetic parameters. The α-half-life varied from 2.2 to 13.8 mins; β-half-life, from 27.1 to 127.6 mins; V_1, from 3.29 to 20.53 L/m^2; V_2, from 6.93 to 72.52 L/m^2; and total body clearance, from 196.3 to 555.6 ml/min/m^2. The patient with the longest half-life (β-half-life) was the only patient with prior exposure to mitomycin.

Using multiple linear regression analyses, we looked for effects of abnormal liver function as assessed by aspartate transaminase, alkaline phosphatase, and total and direct bilirubin, and of abnormal renal function as assessed by creatinine on elimination half-life and total body clearance of mitomycin. No relationships were found.

Figure 1 displays the mean ± SEM plasma concentration of mitomycin following administration of different doses. Patients given 15 mg/m^2 of mitomycin were grouped with patients given 20 mg/m^2. One patient (No. 13, table 1) given 12 mg/m^2 of mitomycin and doxorubicin had a marked prolongation of the β-half-life of mitomycin. He was the only patient treated previously with mitomycin.

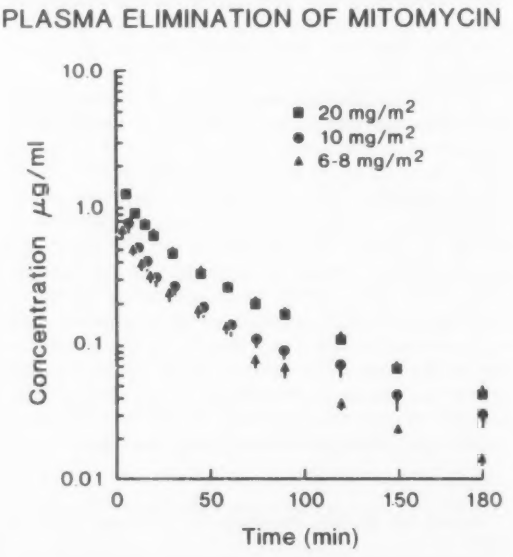

FIGURE 1.—Mean ± SEM mitomycin, C × T data for 12 patients receiving 15-20 mg/m^2, 8 receiving 10 mg/m^2, and 9 receiving 6-8 mg/m^2.

TABLE 1.—Pharmacokinetic parameters of patients receiving mitomycin

Patient No.	Dose (mg/m^2)	Regimen*	$t_{1/2\alpha}$ (mins)	$t_{1/2\beta}$ (mins)	V_1 (L/m^2)	V_2 (L/m^2)	Total body clearance ($ml/min/m^2$)	C × T ($\mu g/min/ml$)	24-hr urinary excretion (% of total dose)
1	20	M	11.9	60.1	15.3	35.5	401.2	49.9	2.2
2	20	M	4.7	36.4	10.4	18.5	448.3	44.6	—
3	20	M	5.4	32.6	14.3	16.7	551.9	36.2	7.4
4	20	M	13.8	61.2	8.9	19.8	216.9	92.2	12.1
5	20	M	4.2	41.0	6.4	23.5	367.3	54.5	17.3
6	20	M	7.7	65.6	10.1	23.8	286.8	69.7	—
7	20	M	4.4	50.2	8.5	33.8	433.1	46.2	19.3
8	20	M	5.6	46.5	9.6	21.1	360.8	55.4	4.7
9	20	M	4.0	61.9	10.2	33.6	406.0	49.3	0.7
10	15	M	10.3	44.3	9.4	23.5	326.0	46.0	—
11	15	M	13.2	62.0	9.8	12.5	196.3	76.4	—
12	15	M	12.2	46.1	8.7	21.0	272.9	55.0	19.9
13	12	MA	13.4	127.6	8.7	72.5	235.9	50.9	1.7
14	10	MT	6.1	52.2	12.0	30.4	433.6	23.1	7.5
15	10	MT	9.9	89.3	20.5	15.4	257.7	38.8	11.4
16	10	MT	13.8	62.9	8.3	36.1	251.3	39.8	2.1
17	10	MT	10.3	63.1	8.2	15.3	197.8	50.6	3.6
18	10	MT	3.3	60.2	4.9	36.1	337.0	29.7	4.2
19	10	FAM	3.4	35.3	3.3	21.3	298.2	33.5	—
20	10	FAM	5.0	47.2	8.8	44.6	508.6	19.7	3.0
21	10	MAP	8.5	42.5	11.4	18.3	367.0	27.2	12.8
22	8	MAP	6.9	48.7	5.8	20.6	250.5	31.9	—
23	8	MAP	4.0	31.1	3.8	6.9	194.7	41.1	—
24†	8	MAP	—	30.8	—	9.4	210.3	38.0	8.0
25	7.5	MCM	6.0	34.6	10.2	33.7	555.6	13.5	—
26†	7.5	MCM	—	27.1	—	10.3	264.1	28.4	—
27†	7.5	MCM	—	32.5	—	13.3	283.5	26.5	—
28	7.5	MCM	12.3	48.3	13.0	54.9	468.6	16.0	—
29	6	MCM	11.2	58.9	8.4	38.6	295.6	20.3	—
30	6	MCM	10.3	56.5	12.8	19.4	309.3	19.4	7.9
MEAN:			8.2	51.8	9.7	26.0	332.9		8.1
SEM:			0.7	3.6	0.7	2.6	19.3		1.4

*M = mitomycin; MA = mitomycin/doxorubicin; MT = mitomycin/Baker's antifol; FAM = mitomycin/doxorubicin/5-FU; MAP = mitomycin/doxorubicin/cisplatin; MCM = mitomycin/lomustine/methotrexate.

†1-compartment model.

The pharmacokinetic data calculated for this patient were not included in the curves of drug elimination shown in figure 1. The summary pharmacokinetic parameters are listed in table 2. The only parameter which differed significantly among levels was the β-half-life (F-test P-value = 0.03). However, a test for trend among mean values was not significant.

The amount of mitomycin eliminated in urine over the first 3 hrs after drug administration was calculated for ten patients, and the amount eliminated over 24 hrs was calculated for eight other patients. Urinary excretion of mitomycin varied from 0.7% to 19.9% of the total dose administered. The lowest urinary excretion of drug was recorded in the patient with a creatinine of 3.1 mg/dl. Six patients had urine collected for the first 3 hrs, and then over the next 21 hrs. In none of these patients was mitomycin detectable in the 21-hr collection. Figure 2 shows the cumulative urinary excretion over 3 hrs in one patient who gave several samples during that period. Approximately 75% of the total excreted in the urine was excreted in the first hour.

DISCUSSION

In this study, we defined the pharmacokinetics of mitomycin in 30 patients. We had previously reported an increase in the half-life of mitomycin in two patients with abnormal serum concentrations of aspartate transaminase (11). This was of interest because extensive clinical experience with mitomycin has never convincingly indicated that modification of dose is necessary in the presence of hepatic or renal disease (12). With further accrual, we have found no differences between the rates of plasma elimination of mitomycin in 17 patients with abnormal liver function, including seven with elevated direct bilirubin in serum and 13 with no abnormalities in standard parameters of liver function.

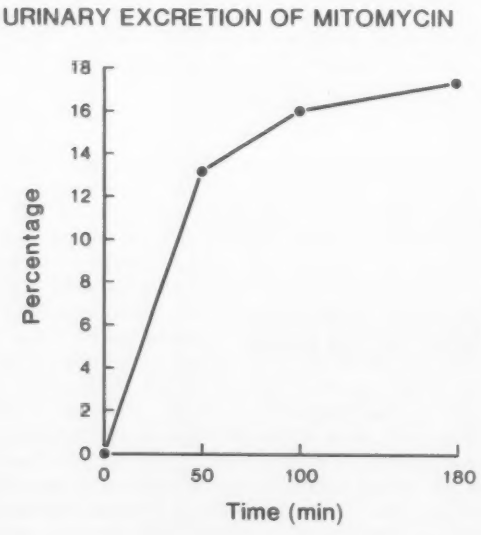

FIGURE 2.—Cumulative urinary excretion of mitomycin over 3 hrs for Patient 5 expressed as percentage of administered dose.

Our results are in general agreement with those of den Hartigh et al (8), Fujita [as recalculated by Reich (13)], and Sarna et al (2). Unlike Fujita, however, we found no evidence of dependence of rate of plasma elimination upon the dose administered within the range of 6–20 mg/m^2. We are cautious in concluding that mitomycin does not show dose-dependent behavior, because all of our patients treated at the highest dose received mitomycin alone, and all patients receiving lower doses were given mitomycin in combination with other drugs. If the other drugs prolonged the elimination of mitomycin, we may not have been able to appreciate dose-dependent behavior. We believe this possibility is unlikely because the rates of elimination of mitomycin given in four different combination regimens were virtually identical. The finding by Sarna et al (2) that there is no difference in the rate of plasma elimination of mitomycin in humans after 20 and 40 mg/m^2 lends further support to the conclusion that mitomycin does not show dose-dependent pharmacokinetic behavior.

Several types of evidence suggest that the cytotoxicity of mitomycin depends upon the total amount of mitomycin reaching a given target and not upon an absolute "threshold" concentration. Barlogie and Drewinko (14) have clearly demonstrated that the cytotoxicity of mitomycin in vitro against the human colon carcinoma cell line LoVo is directly dependent upon concentration times duration of exposure (C × T) and is not dependent upon a given concentration in the culture medium. Clinical experience indicates that, as in vitro, the magnitude of mitomycin cytotoxicity in vivo is not dependent upon a given plasma concentration. Yap et al (15) noted dose-limiting myelosuppression after 15–20 mg/m^2 was given continuously over 5 days, depending upon the extent of prior treatment. Lokich et al (16) administered mitomycin by continuous infusion for 5 days up to 30 days. Bone marrow suppression was dose-limiting at doses ranging from 30 mg for the 5-day infusions to 20 mg for the 30-day infusions. Although it cannot be determined what the C × T values were in these studies, it is certain that peak plasma concentrations were several orders of magnitude lower than when a comparable dose of mitomycin is given by rapid iv injection. In the absence of dependence of cytotoxic activity upon a given threshold concentration, cytotoxicity of mitomycin should be directly related to the magnitude of C × T. Because C ×

TABLE 2.—Summary pharmacokinetic data according to dose of mitomycin

Dose (mg/m^2)	$t_{1/2\alpha}$ (mins)			$t_{1/2\beta}$ (mins)			V_1 (L/m^2)			V_2 (L/m^2)			Clearance ($ml/min/m^2$)		
	15-20	10	6-8	15-20	10	6-8	15-20	10	6-8	15-20	10	6-8	15-20	10	6-8
Mean*	8.1	8.2	8.5	50.5	60.2	41.0	10.1	9.6	9.0	23.6	32.2	23.0	355.6	320.8	314.7
Mean†	7.2	6.4	8.2	47.2	53.9	39.2	11.7	9.1	8.3	20.3	22.3	13.3	371.4	317.8	285.1
Median	6.7	8.5	8.6	48.4	64.5	34.6	9.7	8.7	9.3	22.3	30.4	19.4	364.1	298.2	283.5

*Mean of individual pharmacokinetic parameters from table 1.
†NONLIN fit of data in fig 1.

T is directly dependent upon total dose in the absence of dose-dependent pharmacokinetic behavior, it is reasonable to conclude that the cytotoxic effect of mitomycin, at least with respect to myelosuppression, depends upon total dose and is independent of rate of administration.

The remarkable finding that comparable doses of mitomycin administered over 3 mins or 30 days produce myelosuppression of comparable degrees suggests that the target through which mitomycin induces myelosuppression must undergo cumulative irreversible or slowly reversible alteration in direct proportion to the amount of mitomycin encountered.

It is not known whether the molecule reaching and altering target sites is parent compound or a metabolite of mitomycin. Mitomycin has been reported to be metabolized more rapidly by hepatic tissue than by other tissues in vitro (5,17), but the relationship of hepatic metabolism to the cytotoxic activity of mitomycin is not clear. Tomasz and Lipman (18) found no metabolism of mitomycin using a complete rat liver microsomal metabolizing system in the presence of air. Kennedy et al (19) found some metabolism of mitomycin by murine hepatic microsomes in air, and that optimal biotransformation of mitomycin in vitro required NADPH, NADPH-cytochrome P-450 reductase, phospholipid cytochrome P-450, and an anaerobic environment. Kennedy et al (20) also found that the toxicity of mitomycin against the tumor cell line EMT6 in vitro is maximal under stringent anaerobic conditions. The relevance of these findings to the therapeutic activity of mitomycin in vivo is not clear. Although mitomycin may be maximally cytotoxic under anaerobic conditions, the drug has been demonstrated to be highly cytotoxic in the presence of air to LoVo in exponential- and plateau-phase growth (14). We have documented in eight different human tumor cell lines that LoVo is not unique in its sensitivity to mitomycin in air and in the absence of an exogenous activation system (J. S. Kovach and G. A. van Hazel, unpublished data). At the present time, we believe that, as in vitro, there is no reason to suspect that in vivo mitomycin requires activation at a site other than the target tissue.

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Treatment of Five Subcutaneous Human Glioma Tumor Lines in Athymic Mice With Carmustine, Procarbazine, and Mithramycin[1,2,3]

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Five human glioma tumor lines were transplanted subcutaneously in athymic nude mice. Three of the lines (D-54 MG, U-118 MG, and U-251 MG) were derived from permanent human glioma cell lines, while the other two lines (N-456 and N-519) were established as direct xenografts in mice. Growth rates varied among the lines, but all were treated at a common average tumor volume of 200–300 mm³. Three drugs of known clinical activity against human anaplastic glial tumors were tested in this system. Procarbazine (PCB) produced significant responses in all five tumor lines, carmustine produced significant growth delays in two, and mithramycin produced a slight growth delay in only one. A carmustine-PCB combination produced a greater therapeutic effect than either agent alone against D-54 MG, but the combination was no more effective than PCB alone against U-118 MG. These results parallel clinical experience and indicate that this is a useful model for testing agents of potential activity in brain tumor therapy. [Cancer Treat Rep 67:811–819, 1983]

Anaplastic glial tumors are among the most difficult human neoplasms to treat. Their resistance to conventional forms of therapy is probably based on several factors, including relative radioresistance, impaired antineoplastic drug delivery, and their heterogeneous cellular nature. The search for effective new chemotherapeutic agents against primary human brain tumors has centered on the use of diverse animal tumor models, most of which are histologically distinct from the most common human brain tumors and many of which are of non-neural origin (eg, L1210 leukemia). An in vivo system using human neoplastic glial cells offers many advantages as a system for testing agents of interest in brain tumor chemotherapy. In this paper we are documenting our initial chemotherapy experience with the athymic mouse-human glioma model using carmustine (BCNU), procarbazine (PCB), and mithramycin. The quantitative results indicate that this is a useful system for testing interesting new agents, including those in the preclinical stage of development.

METHODS

Animals.—Homozygous nu/nu, BALB/c athymic mice were used for these experiments. They were produced from an independent breeding colony at Duke University that originated from animals purchased from GIBCO Animal Resources Laboratories (Madison, WI). Animals were maintained in an isolation facility, with their cages, food, and water changed and sterilized weekly. All procedures were performed under sterile conditions; under such conditions, the spontaneous death rate for animals < 1 year of age was < 1%.

Tumor lines.—D-54 MG is the subline of A-172 maintained at Duke University since 1974 and was originally derived from a human glioblastoma multiforme (GBM) (1), while U-118 MG and U-251 MG were established in tissue culture in Uppsala, Sweden, by Professors J. Ponten and B. Westermark. Detailed analysis of biochemical, kinetic, and immunologic traits of these lines has been reported elsewhere (2). Each of these cell lines produces serially transplantable subcutaneous tumors after implantation into athymic mice and each maintained a human glucose-6-phosphate dehydrogenase isoenzyme pattern after serial transplantation in mice (3). N-456 was directly established from a human GBM into the subcutaneous tissue of athymic mice at Duke University (4). Morphologically, it is composed of anaplastic spindle cells that fail to

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[3]Procarbazine (Matulane) was supplied by Hoffmann-LaRoche, Inc, for these studies.

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express glial fibrillary acidic protein (GFAP) after serial animal passage (5). N-519 was established in mice at Duke from a human gliosarcoma. It contains a mixture of small anaplastic cells that express GFAP and a population of spindle cells that are GFAP-negative. Its average subcutaneous tumor volume doubling time is 4.0 days in athymic mice. Chromosomal analysis of mouse-borne N-456 and N-519 performed by Dr. S. Bigner indicated maintenance of a human karyotype and will be reported in detail elsewhere. All tumor lines were treated between a fifth and a tenth animal passage level.

Tumor passage.—For tumor passage, donor animals were killed by cervical dislocation, and their subcutaneous tumors were removed. The tumor was passed through a graded series of sieves and decreasing bore needles to allow injection through a 20-gauge needle. Thirty microliters of tumor were implanted into the right subcutaneous flank of recipient animals using a Hamilton syringe (Hamilton Co, Reno, NV). Thirty to 45 animals received tumor at individual passages.

Drug toxicity.—The lethal toxicity of individual drugs was determined in our colony of animals by probit analysis (6). The dose used in the chemotherapy experiments was 0.75 of the calculated LD10. These doses were: BCNU, 75 mg/m² (~ 25 mg/kg) × 1; PCB, 700 mg/m² (~ 233 mg/kg) daily × 5; and mithramycin, 1.3 mg/m² (~ 0.4 mg/kg) daily × 5. All drugs were administered ip in a volume of 0.01 ml/g of body weight. BCNU was administered in 12% ethanol, while PCB and mithramycin were dissolved in normal saline. The BCNU-PCB combination consisted of PCB at a dose of 700 mg/m² daily for 5 days and BCNU at a dose of 37.5 mg/m² on the first day of treatment. Since animals were always killed before a lethal tumor burden was reached, we considered any spontaneous death after drug treatment to be due to drug toxicity.

Chemotherapy.—After passage, subcutaneous tumors were measured twice weekly with calipers. Tumor volume was calculated according to the formula $a^2 \cdot b/2$, where a = width and b = length (3). When the median tumor volume first exceeded 200 mm³, the animals were divided into groups of ten so that there were no significant differences in average tumor volumes among the groups. One group of ten animals served as a common control and received drug vehicle. Each of the other groups of ten received drug. Drugs were administered ip according to the schedule described previously. Tumors were then measured twice weekly at least until a tumor volume of 1 cm³ was achieved.

Determination of chemotherapeutic response.—Response to drug therapy was measured in two ways. First, the number of tumors regressing to a tumor volume less than the volume at treatment was determined. These tumor regressions were expressed as a fraction of the total number of tumors treated. Statistical significance versus controls was determined by the Fisher exact test. Second, the time from treatment to five times the treatment volume was determined. Exponential growth was assumed, and days from treatment to five times tumor volume at treatment were calculated for individual tumors using linear regression. Statistical significance between control and treated animals was determined by the Wilcoxon rank-sum test. The median difference between the time for individual treated tumors to reach that volume and the median control time was called the median growth delay or T-C.

RESULTS

Growth pattern.—All tumors grew as circumscribed, lobulated subcutaneous masses with a variable tendency to invade surrounding soft tissue structures. The masses fluctuated in size for variable periods after implantation before exponential growth began. In these experiments, each animal that received a tumor implant developed a palpable subcutaneous tumor by the treatment day. The time from transplantation to treatment varied among the lines. D-54 MG was treated on Day 11 in the single-drug experiment and on Day 9 in the combination-drug experiment; U-118 MG on Days 17 and 15, respectively, in the single- and combination-drug experiments; U-251 MG on Day 18; N-456 on Day 20 in the PCB experiment and on Day 26 in the BCNU experiment; and N-519 on Day 19. The average tumor volume at treatment was between 200 and 300 mm³ in all experiments.

Chemotherapy.—There were six animal deaths attributed to drug toxicity in these experiments: two from PCB, one from mithramycin, and three from the BCNU/PCB combination. Each of the drugs produced a significant effect against at least one tumor line, and each of the lines was sensitive to at least one drug (tables 1–4; figs 1–6). There were no significant D-54 MG tumor regressions with any of the three drugs used alone, although each produced statistically significant growth delays. The T-C value for BCNU against D-54 MG was 16.7 days, while for PCB it was 26.2 days. The BCNU-PCB combination produced eight tumor regressions in eight tumors treated, a T-C > 100 days, and six "cures" (no palpable tumor 100 days after treatment). The therapeutic effect produced by this combination was significantly greater than that produced by either drug alone. The modest 2.7-day growth delay produced by mithramycin achieved statistical significance at $P < 0.05$, but this was the only significant effect produced by mithramycin in any of the experiments; this effect could not be repeated in subsequent experiments. U-118 MG was sensitive to PCB (six tumor regressions in ten tumors treated; 21.1-day growth delay) and insensitive to BCNU (no tumor regressions in ten tumors treated; 2.0-day growth delay). The BCNU-PCB combination was also effective against this tumor line, although no more so than PCB alone (three tumor regressions in nine tumors treated; T-C = 23.7 days). BCNU and PCB each produced significant tumor regressions against U-251 MG (seven in ten and

TABLE 1.—BCNU treatment of 5 subcutaneous human glioma tumor lines in athymic mice

	Tumor line				
	D-54 MG	U-118 MG	U-251 MG	N-456	N-519
Control animals					
TD* (days)	2.1 ± 0.3	7.7 ± 2.6	4.1 ± 1.9	4.1 ± 1.0	4.0 ± 1.1
Treatment Day	11	17	18	26	19
Average vol at treatment (mm^3)	243.7 ± 86.6	217.5 ± 35.7	222.1 ± 55.2	249.5 ± 105.8	238.7 ± 135.8
Days from treatment to 5 × treatment vol	4.6 ± 0.8	18.2 ± 5.2	11.2 ± 3.3	9.4 ± 1.8	9.3 ± 2.5
Treatment animals					
Average vol at treatment (mm^3)	250.7 ± 93.6	225.7 ± 51.2	224.6 ± 35.2	249.6 ± 81.3	248.6 ± 113.4
Days from treatment to 5 × treatment vol (significance vs control)†	21.6 ± 4.8 ($P < 0.01$)	20.0 ± 2.5 (NS)‡	19.1 ± 2.7 ($P < 0.05$)	9.3 ± 1.9 (NS)	10.7 ± 1.6 (NS)
T-C§	16.7	2.0	8.1	-1.3	2.3
No. of tumor regressions/ No. treated	0/10	0/10	7/10¶	0/10	0/10
No. of toxicity deaths/ No. treated	0/10	0/10	0/10	0/10	0/10

*TD = tumor volume doubling time.
†Wilcoxon rank-sum test.
‡NS = not significant ($P > 0.05$).
§Median difference in days to 5 × treatment volume between treated and control animals.
¶$P < 0.05$ vs control by Fisher exact test.

TABLE 2.—PCB treatment of 5 subcutaneous human glioma tumor lines in athymic mice

	Tumor line				
	D-54 MG	U-118 MG	U-251 MG	N-456	N-519
Control animals					
TD* (days)	2.1 ± 0.3	7.7 ± 2.6	4.1 ± 1.9	3.3 ± 0.5	4.0 ± 1.1
Treatment Day	11	17	18	20	19
Average vol at treatment (mm^3)	243.7 ± 86.6	217.5 ± 35.7	222.1 ± 55.2	227.2 ± 47.9	238.7 ± 135.8
Days from treatment to 5 × treatment vol	4.6 ± 0.8	18.2 ± 5.2	11.2 ± 3.3	7.1 ± 1.4	9.3 ± 2.5
Treatment animals					
Average vol at treatment (mm^3)	245.3 ± 107.8	218.9 ± 38.0	222.8 ± 32.7	257.0 ± 68.5	243.4 ± 122.0
Days from treatment to 5 × treatment vol (significance vs control)†	31.1 ± 1.7 ($P < 0.01$)	41.6 ± 7.0 ($P < 0.01$)	85.8 ± 39.4 ($P < 0.01$)	12.4 ± 2.1 ($P < 0.01$)	24.0 ± 2.9 ($P < 0.01$)
T-C‡	26.2	21.1	64.7	6.1	14.3
No. of tumor regressions/ No. treated	1/10	6/10§	8/8¶	0/10	8/10§
No. of toxicity deaths/ No. treated	0/10	0/10	2/10	0/10	0/10

*TD = tumor volume doubling time.
†Wilcoxon rank-sum test.
‡Median difference in days to 5 × treatment volume between treated and control animals.
§$P < 0.05$ vs control animals by Fisher exact test.
¶$P < 0.01$ vs control by Fisher exact test.

TABLE 3.—Mithramycin treatment of 3 subcutaneous human glioma tumor lines in athymic mice

	Tumor line		
	D-54 MG	U-251 MG	N-519
Control animals			
TD* (days)	2.1 ± 0.3	4.1 ± 1.9	4.0 ± 1.1
Treatment Day	11	18	19
Average vol at treatment (mm^3)	243.7 ± 86.6	222.1 ± 55.2	238.7 ± 135.8
Days from treatment to 5 × treatment vol	4.6 ± 0.8	11.2 ± 3.3	9.3 ± 2.5
Treatment animals			
Average vol at treatment (mm^3)	248.2 ± 110.4	222.4 ± 52.6	242.2 ± 131.8
Days from treatment to 5 × treatment vol (significance vs control)†	7.1 ± 1.4 ($P < 0.05$)	10.6 ± 3.1 (NS)‡	12.2 ± 3.0 (NS)
T-C§	2.7	-0.2	2.4
No. of tumor regressions/ No. treated	0/10	0/10	0/9
No. of toxicity deaths/ No. treated	0/10	0/10	1/10

*TD = tumor volume doubling time.
†Wilcoxon rank-sum test.
‡NS = not significant ($P > 0.05$).
§Median difference in days to 5 × treatment volume between treated and control animals.

eight in eight, respectively). BCNU produced a T-C of 8.1 days, while the T-C value for PCB against U-251 MG (64.7 days) was the largest response to a single agent in this series of experiments, although no cures were achieved. Mithramycin was ineffective against U-251 MG. The two directly transplanted tumor lines showed similar patterns of drug sensitivity. Neither was sensitive to BCNU, but PCB produced significant growth delays in each. The magnitude of the delay produced by PCB was greater in N-519 (14.3 vs 6.1 days). Mithramycin was ineffective against N-519.

DISCUSSION

In these experiments, we have documented a variable response of human neoplastic glial cells growing in athymic mice to BCNU, PCB, and mithramycin. BCNU produced significant growth delays in D-54 MG and U-251 MG, although only in U-251 MG was there a significant number of tumor regressions. The effect of BCNU on U-118 MG was less than reported previously (3), possibly because the treatment protocol had been modified and the tumors were somewhat larger at the time of treatment. PCB had a statistically significant effect against all five of the lines, although the magnitude of the response varied from a growth delay of 6.1 days and no tumor regressions in ten N-456 tumors treated to a growth delay of 64.7 days and eight tumor regressions in eight U-251 MG tumors treated. A BCNU-PCB combination was highly effective against D-54 MG, producing six cures in ten tumors 100 days after treatment. However, this combination was no more effective than PCB alone against U-118 MG. Mithramycin produced a significant growth delay only against D-54 MG, and even in this case the magnitude of the delay (2.7 days) was small.

These three agents were chosen for the initial chemotherapy experiments in this system because their activity against human anaplastic gliomas has been documented. BCNU has produced response rates of 23%-36% in recurrent gliomas (7,8), and it has significantly increased the number of 18-month survivors when combined with radiotherapy immediately after surgery for malignant glioma (9). Kumar et al (10) documented a 32% response rate among 25 patients with recurrent gliomas treated with PCB, and the Brain Tumor Study Group (BTSG) has recently reported that PCB was as effective as BCNU in prolonging survival of patients with malignant glioma (11). A clinical BCNU-PCB combination has not been used extensively, although Levin et al (12) reported a 29% response rate and a median response duration of 34 weeks. An alternating BCNU-PCB regimen is an arm of the current BTSG protocol so that additional clinical information will soon be available. Mithramycin, on the other hand, is ineffective as a single agent against gliomas. In the first BTSG randomized study of chemotherapy for brain tumors, treatment with mithramycin was no

TABLE 4.—BCNU + PCB treatment of 2 subcutaneous human glioma tumor lines in athymic mice

	Tumor line	
	D-54 MG	U-118 MG
Control animals		
TD* (days)	2.5 ± 0.5	5.5 ± 1.1
Treatment Day	9	15
Average vol at treatment (mm^3)	270.5 ± 16.5	262.3 ± 63.1
Days from treatment to 5 × treatment vol	6.2 ± 1.1	15.7 ± 2.6
Treatment animals		
Average vol at treatment (mm^3)	270.7 ± 92.1	263.8 ± 67.6
Days from treatment to 5 × treatment vol (significance vs control)†	> 100 ($P < 0.01$)	39.7 ± 10.2 ($P < 0.01$)
T-C‡	> 100	23.7
No. of tumor regressions/ No. treated	8/8§	3/9¶
No. of toxicity deaths/ No. treated	2/10	1/10

*TD = tumor volume doubling time
†Wilcoxon rank-sum test.
‡Median difference in days to 5 × treatment volume between treated and control animals.
§$P < 0.01$ vs controls by Fisher exact test.
¶$P < 0.01$ vs controls by Fisher exact test.

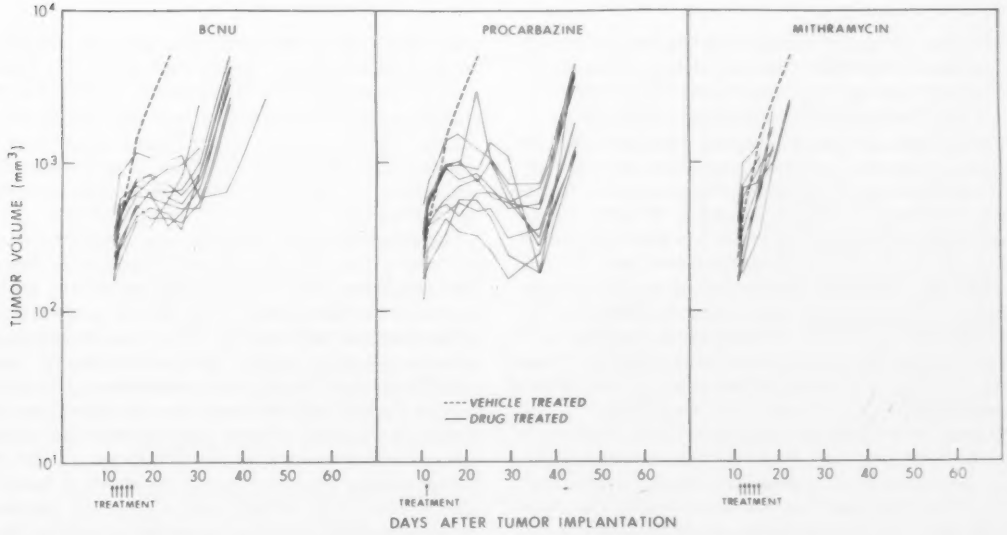

FIGURE 1.—Response of the human glioma-derived cell line D-54 MG in athymic mice to BCNU, PCB, and mithramycin. Drugs were administered ip beginning on Day 11 at 0.75 of their LD10. Dashed line represents the median growth curve of the 10 control tumors. Solid lines represent individual growth curves of treated tumors.

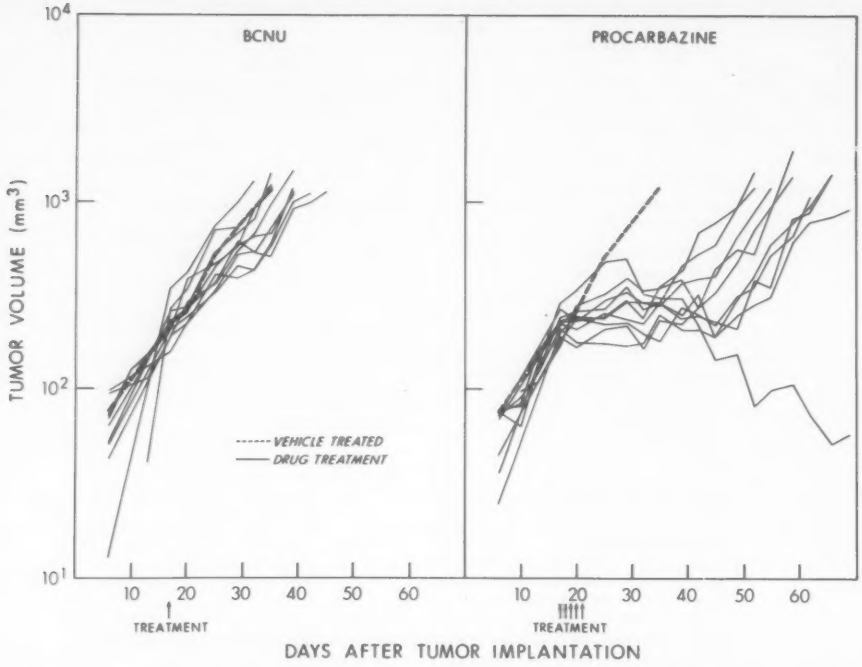

FIGURE 2.—Response of the human glioma-derived cell line U-118 MG in athymic mice to BCNU and PCB. Conditions were identical to those in the D-54 MG experiment except that the drugs were administered beginning on Day 17.

better than no further therapy (13). The chemotherapeutic results in this system therefore parallel clinical experience, although our data suggest that PCB is somewhat more effective than BCNU under these conditions.

Even though the results in general reflected clinical experience, there was considerable variability in sensitivity to these three agents among the five tumor lines. Variability of therapeutic responses among different human tumors of the same histologic type has been documented repeatedly using both immunosuppressed mice (14) and congenitally athymic nude mice (15,16), and it is not surprising in the biologically heterogeneous GBM (17). Presumably individual tumor lines exhibit characteristic and to some extent unique patterns of drug sensitivity. However, until the biologic basis of resistance to drug effects is understood, such patterns remain descriptive.

Despite the variability among tumor lines, therapeutic results in athymic mice have relevance for human gliomas for a number of reasons. The neoplastic cells were derived from gliomas, they resemble neoplastic glia morphologically and, in some cases, immunohistochemically, and their experimental drug sensitivity parallels clinical experience. Even though cellular selection may have occurred in the established cell lines and, perhaps to a lesser extent, in the transplanted xenografts (18), the selected population is likely to be the most biologically aggressive and therefore clinically relevant population. The sensitivity mosaic of a battery of such glial neoplasms of human origin in athymic mice will have implications for the choice of appropriate drugs for clinical evaluation, the design of alternative combinations and schedules, and patterns and mechanisms of cross-resistance.

The subcutaneous location also has many advantages for testing the chemotherapeutic sensitivity of human glial neoplasms. Pharmacologic uncertainties of testing in vitro are avoided since, in the animal, drugs must be metabolized and delivered, and there must be acceptable toxicity to normal organs. One can accumulate serial quantitative data rapidly with measurements of subcutaneous tumors, and the results can be analyzed and expressed in a number of ways. In an intracerebral model, only one end-point, survival, can be analyzed. Finally, the subcutaneously implanted tumor allows one to focus on the question of the cellular sensitivity of the neoplasm while temporarily avoiding important questions about the brain microenvironment and drug delivery to an intracerebral tumor. These questions can be addressed directly in separate experiments since cells from human

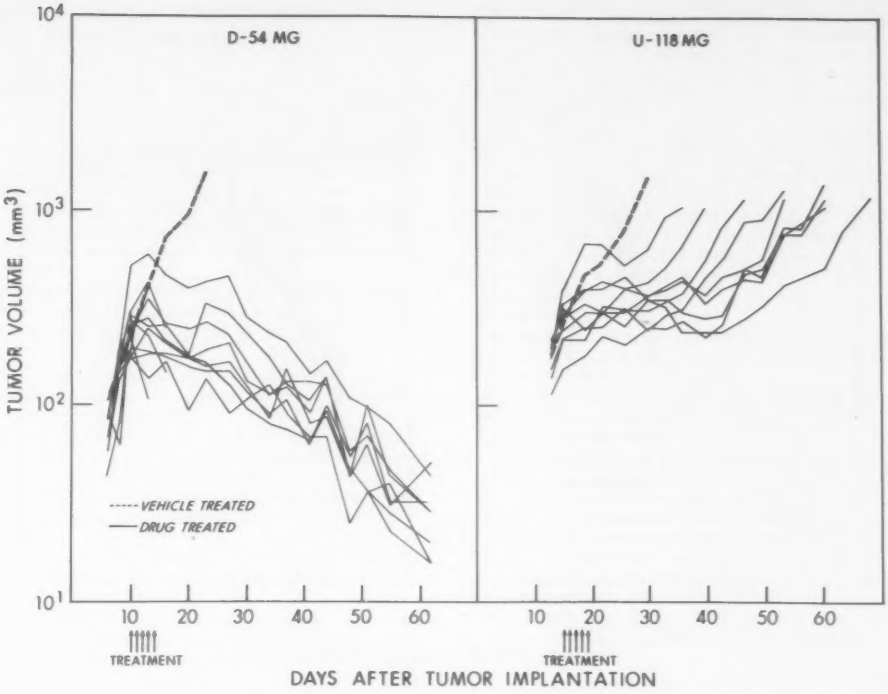

FIGURE 3.—Response of D-54 MG and U-118 MG to a combination of BCNU and PCB. BCNU was administered on the 1st treatment day, and PCB was administered daily × 5. There was a synergistic effect on D-54 MG with > 50% cures, while the combination was no more effective against U-118 than PCB alone.

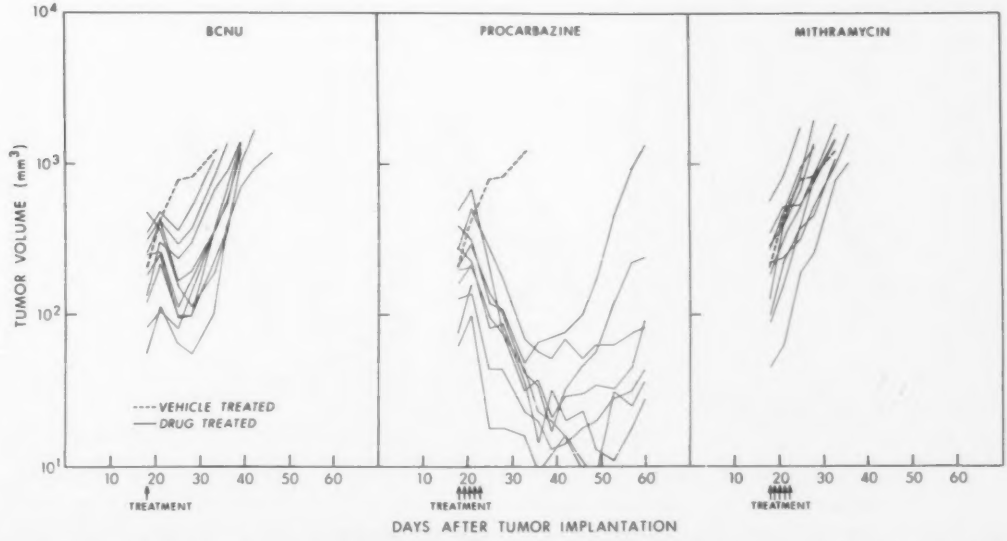

FIGURE 4.—Response of the human glioma-derived cell line U-251 MG in athymic mice to BCNU, PCB, and mithramycin. Median growth delay after PCB treatment exceeded 60 days, but all tumors recurred.

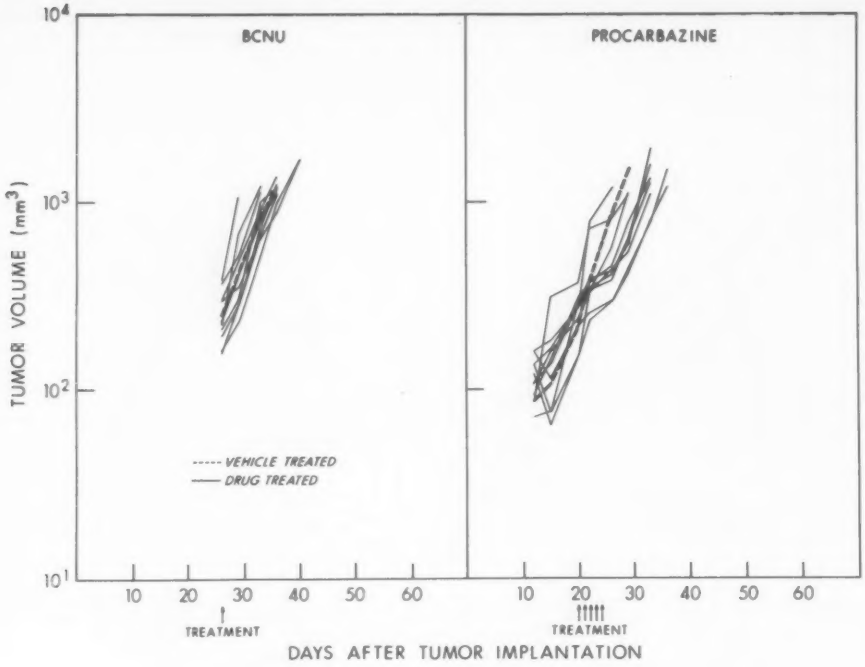

FIGURE 5.—Response of the human glioblastoma xenograft N-456 to BCNU and PCB.

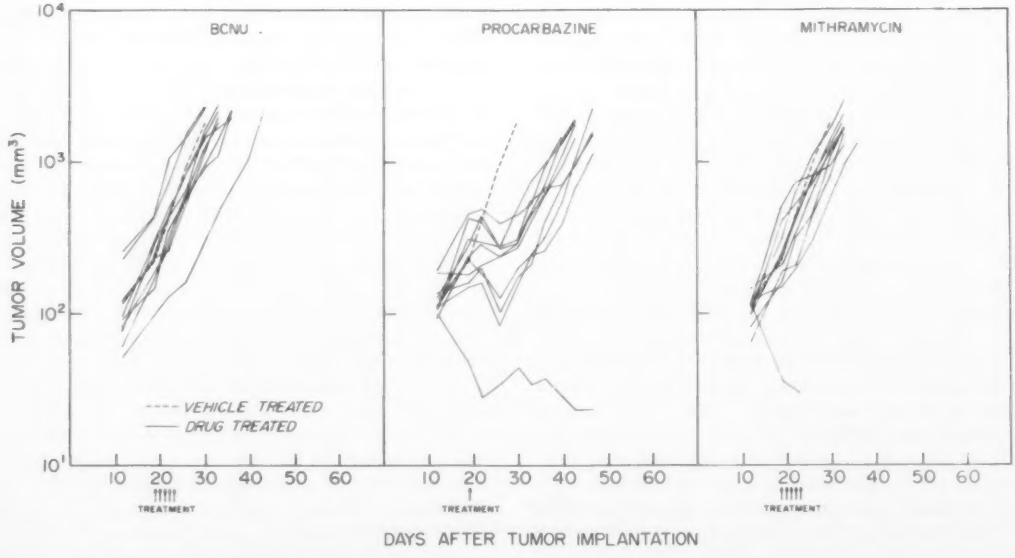

FIGURE 6.—Response of the human gliosarcoma xenograft N-519 to BCNU, PCB, and mithramycin.

gliomas also grow well after intracerebral implantation in the athymic mouse (3,19).

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Vindesine in the Treatment of Malignant Mesothelioma: A Phase II Study[1,2]

David Kelsen,* Richard Gralla, Edgar Cheng, and Nael Martini[3]

Vindesine (VDS) is a new semisynthetic vinca alkaloid which has demonstrated therapeutic activity against a variety of solid tumors. A phase II trial of this agent was performed in patients with advanced malignant mesothelioma. VDS at a dose of 3 mg/m² was given iv once weekly for 4–6 weeks and then every 2 weeks thereafter. Only one of 17 evaluable patients had a partial remission, lasting 5 months (95% confidence limits, 0%–17%). Toxic effects of VDS included leukopenia, peripheral neuropathy, and alopecia. VDS has minimal activity in malignant mesothelioma. [Cancer Treat Rep 67:821–822, 1983]

Vindesine (VDS) is a new semisynthetic vinca alkaloid that has shown activity in a variety of in vitro tumor models (1). Phase I studies indicated that doses of 3–4 mg/m²/week were appropriate for further clinical trials. In phase II studies, the drug has induced objective responses in patients with non-small cell lung cancer (2), melanoma (3), breast cancer (3), and esophageal carcinoma (4). In combination with other agents, higher response rates were seen in patients with lung and esophageal carcinomas (5,6). Because of its encouraging activity against other tumors, a phase II trial in patients with malignant mesothelioma was undertaken at the Memorial Sloan-Kettering Cancer Center (MSKCC).

MATERIALS AND METHODS

Twenty patients with malignant mesothelioma were entered in this study beginning in July 1977 and ending in December 1981. For all patients, the diagnosis was confirmed by the Department of Pathology, MSKCC. Characteristics of the patient population are shown in table 1. Six patients had received prior chemotherapy. Primary tumor sites included pleural disease in 17 patients, pericardial disease in two, and peritoneal disease in one. All patients had measurable or evaluable disease, with sites of metastases including pulmonary parenchyma in eight, pleural-based masses in ten, and abdominal masses in two. Pulmonary parenchymal lesions were measurable by chest x-ray. Pleural-based masses were considered evaluable by chest x-ray in eight patients and by computerized tomography in two. The abdominal masses were evaluated by physical examination in one patient and by computerized tomographic scan in the other.

VDS was given once weekly for four to six doses and then biweekly until disease progression, or until toxicity (usually neurotoxicity) became severe enough to warrant its discontinuance. A minimum of three doses represented an adequate trial. VDS was given as a rapid iv injection through the side arm of a running iv line. The starting dose was 3 mg/m²/week, with an attenuation schedule as follows: 100% of planned dose was given if the wbc count was $\geq$ 3000/mm³, if the platelet count was $\geq$ 100,000/mm³, and if there was mild to no neurotoxicity; half-dose (1.5 mg/m²) was given if the wbc count was $\geq$ 2000 but $<$ 3000/mm³, if the platelet count was $\geq$ 75,000 but $<$ 100,000/mm³, or if there was moderate to moderately severe neurotoxicity; and VDS was withheld if the wbc count was $<$ 2000/mm³, if the platelet count was $<$ 75,000/mm³, or if severe neurotoxicity was present.

A complete remission was defined as 100% disease regression for at least 1 month. A partial remission was defined as at least a 50% but $<$ 100% decrease in the sum of the two longest perpendicular diameters of clearly measurable lesions, for at least 1 month. A minor regression was defined as a $\geq$ 25% but $<$ 50% decrease in tumor size. In patients with evaluable disease, at least a 75% tumor regression was required to be classified as a response. In all patients, no new lesions could appear during the remission period. If there was no change in the

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	20
Evaluable	17
Median age in yrs (range): 64 (43–71)	
Median performance status (range): 70 (40–90)	
Primary site	
Pleural	17
Pericardial	2
Peritoneal	1
Males:females	13:7
Prior chemotherapy	6

size of the lesions, the patient was considered to be a nonresponder. Informed consent was obtained from all patients prior to the initiation of therapy.

RESULTS

Of the 20 patients entered in the study, 17 were evaluable for response and toxicity. The median number of VDS doses was five. One patient with multiple pulmonary metastases had a partial remission of 5 months' duration (one of 17 patients, 5.9%; 95% confidence limits, 0%–17%). There was one minor response (1 month). One patient had stable disease for 48+ months. The other 14 patients experienced disease progression. The major toxic effects noted included peripheral neuropathy, which was seen in almost all patients but which was severe in only two; alopecia, seen in 80%; and constipation, seen in 25%. Myelosuppresion was mild, and primarily manifested as leukopenia, with a median wbc count nadir of $2.8 \times 10^3/mm^3$. There were no drug-related deaths.

DISCUSSION

Malignant mesothelioma remains a highly lethal disease. Even for those patients who present with disease apparently localized to the hemithorax, resection rates are poor and long-term survival is unsatisfactory, although recent studies suggest that aggressive surgery and radiation therapy, used in combination, have had some impact on the disease (7–9). As is the case for a number of other malignancies (testicular, ovarian, head and neck, and esophageal cancers), multimodality approaches involving the surgeon, radiation therapist, and medical oncologist will probably be necessary to significantly improve the prognosis for this disease. However, this presumes the development of effective chemotherapeutic regimens. Unfortunately, few antineoplastic agents have been studied in this disease and even fewer have demonstrated therapeutic activity (7,10).

Doxorubicin is generally accepted to be the most active of the conventional agents but has been reported to yield major objective regressions in only 20%–25% of patients (11). More recently, an Eastern Cooperative Oncology Group trial has indicated even more modest activity, with only seven of 51 patients responding to doxorubicin as a single agent (12). Thus, there is clearly a need to develop other effective drug regimens.

Although VDS had shown antineoplastic activity in several relatively refractory tumors (non-small cell lung cancer and esophageal carcinoma), our results in the current trial are disappointing, especially in view of the substantial percentage of patients (67%) who had not received prior chemotherapy. There are no data on the use of other vinca alkaloids in this disease. In view of the minimal activity seen with VDS in the current study, we do not plan further trials with this agent in mesothelioma.

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Gallium Nitrate in Malignant Lymphoma: A Southwest Oncology Group Study[1,2]

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Gallium nitrate was evaluated by the Southwest Oncology Group in 38 patients with malignant lymphoma, both Hodgkin's disease and non-Hodgkin's lymphoma. Treatment was administered every 2 weeks at a dose of 700 mg/m², with acceptable toxicity. Seven partial responses (18%) with a duration of 3–11 months were seen: one of seven in Hodgkin's disease and six of 26 in diffuse non-Hodgkin's lymphoma. No responses were noted in nodular types. Gallium nitrate has activity in lymphomas and should be combined with more active drugs for patients who have undergone less intensive prior therapy. [Cancer Treat Rep 67:823–825, 1983]

Gallium citrate has long been established as a diagnostic tool for the localization of both neoplastic lesions and inflammatory disease in man (1). Gallium nitrate, a 3A metal salt, was chosen for clinical trials on the basis of its activity against Walker 256 carcinosarcoma (2). The mechanism by which gallium localizes in tumors is largely unknown. It is not specific for neoplasms, since numerous inflammatory lesions can concentrate isotopic gallium (3). As a result of preclinical trials, phase I and II studies have been conducted at several centers (4–8). Routes and schedules used included bolus administrations at 2–3-week intervals, daily doses for 3 consecutive days, and 7-day continuous iv infusions as well as weekly rapid iv infusions. For this study (SWOG 7912), a dose schedule of 700 mg/m² at 2-week intervals was chosen because of dose-limiting nephrotoxicity at single doses exceeding this value.

PATIENTS AND METHODS

Between May 1979 and May 1981, 38 patients (18–76 years old; median, 49) were treated via SWOG 7912. Patients with either Hodgkin's disease (HD) or non-Hodgkin's lymphoma (NHL) were eligible for this study with no age restrictions. All patients had received prior extensive therapy as shown in table 1. Thirty-two patients had a Southwest Oncology Group (SWOG) performance status of 2 or better (Karnofsky, 50–100), and six had a performance status of 3 or 4 (Karnofsky, 10–40). Measurable disease, adequate renal function (BUN < 20 mg/100 ml and/or creatinine < 1.2 mg/100 ml), and adequate liver function (bilirubin < 2 mg/100 ml and no clinical or chemical evidence of biliary obstruction) were required. A wbc count of $> 3 \times 10^9$/L and a platelet count of $> 100 \times 10^9$/L were the minimum counts required for beginning therapy in the absence of marrow involvement. No concomitant chemotherapy or radiotherapy was allowed and therapy was not permitted for 4 weeks prior to gallium nitrate usage. The treatment regimen was as follows: gallium nitrate was given as a 30-minute infusion at a dose of 700 mg/m² every 2 weeks. Within the 12 hours prior to infusion, all patients were given 2 L of fluid in excess of normal intake, either orally or iv. An additional 500 ml of normal saline was infused in the 2 hours preceding the 30-minute infusion of gallium nitrate. If myelosuppression was noted on Day 14, subsequent courses were withheld until recovery to a wbc count of $> 3 \times 10^9$/L and to a platelet count of $> 100 \times 10^9$/L, or to achievement of the pretreatment values in patients with bone marrow involvement.

Complete remission was defined as the disappearance of all evidence of disease and related symptoms for a minimum of 1 month. An adequate trial was considered to be two courses of therapy. Partial remission was defined as a $\geq 50\%$ reduction in the sum of the product

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TABLE 1.—Characteristics of patients receiving gallium nitrate

Histologic type	No. of patients	Mean No. of prior chemotherapy combinations (range)	Response	
			No.	Duration (mos)
HD				
Nodular sclerosis	5	4.0 (2-7)	1	8
Lymphocyte depleted	2	2,4	—	—
Total	7			
NHL, diffuse				
Histiocytic	10	3.3 (1-6)	2	5,5
Poorly differentiated	6	4.0 (2-5)	2	3,4
Mixed	6	2.0 (2-3)	2	3,11
Well-differentiated	2	1,6	0	—
Undifferentiated	2	2,5	0	—
Total	26			
NHL, nodular				
Poorly differentiated	3	2,3,3	0	—
Mixed	1	4	0	—
Histiocytic	1	5	0	—
Total	5			

of the diameters of measured lesions, lasting for at least 1 month. No simultaneous increase in the size of any lesion or the appearance of new lesions could occur. Patients were removed from the study if increasing disease was noted after two courses of therapy and/or if unacceptable toxicity developed.

RESULTS

Thirty-three of the 38 patients were fully evaluable for response, toxicity, and survival, whereas five were only partially evaluable because death occurred 4-13 days after the start of therapy. Two patients died with HD. Three others with diffuse NHL died, two of pneumonitis uncomplicated by hematologic or renal toxicity. One patient who died of gastrointestinal bleeding had a pretreatment platelet count of only 35 × 10^9/L and a positive bone marrow sample. In two other patients who died at 4 and 13 days, respectively, increasing serum creatinine levels and renal parenchymal lymphoma were found in autopsy specimens. No autopsy material is available for the two patients who died of pneumonitis; in each case pretreatment chest films were consistent with infectious processes. There was no suggestion of a causal relationship between drug administration and subsequent death. Twenty-two men and 16 women were treated: seven with HD, five with nodular NHL, and 26 with diffuse NHL (table 1). These 38 patients received 166 courses, for an average of 4.4 courses each (range, one to 16 courses). Table 1 also shows the average number of prior combination chemotherapy programs undergone by each patient.

There were seven (18%) partial responders (table 1). The duration of response varied from 3 to 11 months; three patients improved but did not meet the criteria for partial response. Twenty-three patients had either no response or exhibited progressive disease on completion of the trial. The response rate is thus 18% (seven of 38 eligible patients), with 21% (seven of 33 patients) being fully evaluable, excluding early deaths. By disease category the response rates for evaluable patients are: HD, 20% (one of five); nodular NHL, 0% (none of five); and diffuse NHL, 26% (six of 23).

Interestingly, of the seven responders only three had responded to prior chemotherapy programs (one each with diffuse mixed lymphoma, diffuse histiocytic lymphoma, and diffuse poorly differentiated lymphoma), whereas the remaining four responders achieved less than a complete remission with standard chemotherapy/radiotherapy programs (mechlorethamine, vincristine, prednisone, and procarbazine [MOPP]-bleomycin in HD and cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]-bleomycin in diffuse histiocytic lymphoma, diffuse and mixed lymphoma, and diffuse poorly differentiated lymphoma). Sites of disease in responders were lymph nodes in all seven, liver in two, lung in one, and skin in one.

Toxicity of this program is shown in tables 2 and 3. No toxic effects were noted for ten of 32 evaluable patients. Hypocalcemia was not seen in 29 of the 32 patients tested; magnesium levels were not obtained. No documentation of decreasing hemoglobin was elicited in any patient in the absence of intestinal bleeding (three patients).

TABLE 2.—Toxicity of gallium nitrate in 32 evaluable patients

	Degree				
	None	Mild	Moderate	Severe	Life-threatening
Leukopenia	10	2	1	1	0
Thrombocytopenia	10	1	2	1	1
Nausea and vomiting, diarrhea	10	6	2	1	0
Renal toxicity	10	1	2	1	1

TABLE 3.—Chemotherapy toxicity criteria

	Degree				
Toxicity	None	Mild	Moderate	Severe	Life-threatening
Wbc count (cells/μl) (granulocytes/μl)	> 4000 (> 1500)	3000-3999 (< 1500)	2000-2999 (< 1000)	1000-1999 (< 500)	< 1000 (< 250)
Platelet count (cells/μl)	≥ 100,000	75,000-99,999	50,000-74,999	25,000-49,999	< 25,000
Diarrhea	< 3 bowel movements/day	3-4 liquid stools/day, no dehydration	> 4 liquid stools/day, needs iv to hydrate	Bloody diarrhea, needs iv and/or blood	
Nausea and vomiting	Normal	Nausea, no vomiting	Vomiting can be prevented by treatment to < 6 × day	Vomiting > 6 × day despite anti-emetics	
Creatinine clearance	> 50	40-50	30-40	20-30	< 20

Hematologic toxic effects (wbc and/or platelet counts) of moderate to life-threatening severity were noted in five patients (both leukopenia and thrombocytopenia were seen in only one person). Pretreatment counts were compromised in three patients with marrow involvement by tumor (platelet counts of 105 × 10^9/L and 50 × 10^9/L and total wbc count of 3.4 × 10^9/L) and were normal in two (platelet count of 300 × 10^9/L and wbc count of 5 × 10^9/L). During therapy, there were two reports of diminished vision which corrected spontaneously within 3 months. No ophthalmologic abnormalities were detected in either patient. In both instances, the medication was being given iv and the problem occurred during the third course.

DISCUSSION

In this group of patients with intense prior therapy, a response rate of 18% with minimal hematologic toxicity and predictable renal toxicity is acceptable. The responses seen in this study using a biweekly schedule of 700 mg/m^2 are similar to those reported by Warrell et al (8), whose 32 patients with measurable disease treated at a daily dose of 200-400 mg/m^2/day had a 43% response rate lasting from 1+ to 9+ months. In this latter report, the response rates in diffuse disease were greater than those reported for the nodular types. Both that and the current SWOG trial show that, as administered, the drug is well-tolerated and that further trials in combination with more active drugs in patients with less intensive prior treatment are warranted.

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Radiation as Salvage Therapy for Patients With Hodgkin's Disease Relapsing After MOPP (Mechlorethamine, Vincristine, Prednisone, and Procarbazine) Chemotherapy[1,2]

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Six patients with Hodgkin's disease who failed MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) chemotherapy, with recurrences confined to lymph node areas, are reported. All patients were treated with tumoricidal doses of irradiation in mantle, inverted Y, or whole-abdominal fields. All six patients achieved complete remission, with minimal toxicity. Disease-free survival ranged from 3 to 38 months, with four patients remaining in complete remission at 9, 15, 27, and 38 months. Radiation therapy should be considered in patients failing MOPP chemotherapy with lymph node disease. [Cancer Treat Rep 67:827–829, 1983]

MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) chemotherapy results in complete remission (CR) in 80% of patients with advanced-stage Hodgkin's disease (HD) (1). However, 30% of the patients who achieve a CR will relapse (1). One-half of these relapsing patients have disease confined to lymph node areas (2). Most oncologists treat such patients with a second course of MOPP or chemotherapy that is non-cross-resistant with MOPP. A review of these regimens shows CR rates from 4% to 60%, short follow-up, and variable use of maintenance chemotherapy and radiation therapy (3). In an attempt to identify agents that are useful in the treatment of relapsed HD, we studied the use of radiation therapy as a single modality. Since radiation is not a systemic therapy, we studied only those patients who had disease recurrence confined to lymph node areas. We report our experience with six patients who, having failed MOPP with disease confined to lymph node areas, received irradiation in an attempt to eradicate all known disease.

MATERIALS AND METHODS

Six patients were selected for the study. They met the following criteria: (*a*) HD had recurred while the patient was receiving or after the patient had received MOPP or MVPP (mechlorethamine, vinblastine, procarbazine, and prednisone); (*b*) the recurrence was confined to lymph node areas that could be encompassed in an irradiation field; (c) irradiation was given to a minimum of mantle or inverted Y in tumoricidal doses; and (*d*) measurable disease was present. Table 1 shows the pretreatment characteristics of these patients. Disease recurrence was established by biopsy (Patients 2, 3, 4, and 6) or by regrowth in sites of known disease (Patients 1 and 5). CR was defined as the total disappearance of disease at all known sites and no new sites of disease. Evaluation of response was based on physical examination (Patients 1, 2, 4, and 5), chest x-ray (Patients 1 and 6), or abdominal computerized axial tomography (Patients 3 and 5). Duration of response was measured from completion of radiation. Patient 2 (stage IIA) was treated with chemotherapy since she was 7 months pregnant at the time of diagnosis.

RESULTS

Table 1 lists the results of treatment. All six patients achieved CR. Four patients remain in continuous CR at 9, 15, 27, and 38 months after completing radiation therapy. Patient 1 relapsed 3 months after radiation therapy and died despite extensive second-line chemo-

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[2]The opinions or assertions expressed here are the private views of the authors and are not to be construed as official or as representing the Department of the Army or the Department of Defense.

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TABLE 1.—Characteristics and response of MOPP-failure patients treated with irradiation

Patient No.	Initial stage	Histologic diagnosis*	Initial therapy†	Initial relapse-free survival (mos)	Site of relapse	Salvage radiation therapy	Response	Relapse-free survival (mos)	Status
1	IIB	NS	MVPP	0	Cervical Mediastinal	Mantle	CR	3	Dead
2	IIA	NS	MOPP	30	Supraclavicular	Mantle	CR	38	No evidence of disease
3	IIA	MC	RT (IF)	12					
		MC	BOPP	36	Multiple				
		MC	MOPP	48	Periaortic				
		MC	AD, CTX	14	Periaortic	Inverted Y	CR	27	No evidence of disease
4	IIIA	MC	MOPP	0	Cervical	Mantle	CR	15	No evidence of disease
5	IIIA	NS	MOPP	0	Periaortic	Whole-abdominal	CR	10	Alive with disease
6	IVA	NS	MOPP	72					
			MOPP	60	Mediastinal	Total-nodal	CR	9	No evidence of disease

*NS = nodular sclerosing; MC = mixed cellular.

†RT (IF) = radiation therapy (involved-field); AD = doxorubicin; CTX = cyclophosphamide; and BOPP = carmustine, vincristine, prednisone, and procarbazine.

therapy. Patient 5 has relapsed and is now receiving second-line ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) chemotherapy. Toxicity has been mild, with the only significant toxic effects being prolonged thrombocytopenia in Patient 5, who received whole-abdominal irradiation, and mild wet desquamation of the skin in Patient 4.

DISCUSSION

Recurrence of HD after treatment with MOPP chemotherapy is treated with a variety of chemotherapeutic regimens. CR rates vary from 4% to 60% and durations of CR are usually short (3). In treating MOPP failures, most studies include combinations of agents that are non-cross-resistant with MOPP. Very few data are available for irradiation alone, the most active single agent in the treatment of HD.

Previous experience with irradiation of patients who failed MOPP chemotherapy is limited. Heusinkveld et al (4) reported six patients who received total-nodal irradiation and one who received mantle irradiation to control lymph node recurrences which occurred after MOPP chemotherapy. They achieved five CRs lasting from 12 to 32+ months. At the time of their report, two patients were still in CR at 29 and 32 months. Portlock et al (5), in a review of salvage treatment of HD, noted three of four CRs in patients receiving irradiation after failing chemotherapy. No further details are provided.

Two warnings about the general applicability of these results must be stated. All of our patients and those of Heusinkveld et al had disease confined to lymph nodes. Additionally, three of our patients had prolonged disease-free intervals following MOPP chemotherapy. Both of these factors have been identified by Straus et al (6) as good prognostic factors. At least part of the successful outcome can be attributed to selection of patients with these two favorable prognostic factors. Concern is also raised by the results in irradiation-failure patients who were re-treated with irradiation. Experience with a large group of these patients at Stanford indicates that there is a slow but steady relapse rate in patients who are re-irradiated after failing irradiation (7). Despite these cautions, our data suggest that irradiation of patients failing MOPP chemotherapy produces high CR rates and prolonged disease-free survival with minimal toxicity. Radiation therapy, either alone or in combination with non-cross-resistant chemotherapy, should be considered for patients failing MOPP chemotherapy with lymph node disease.

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Lack of Efficacy of Vinblastine and Cisplatin in Patients With Progressive Small Cell Carcinoma of the Lung[1]

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Twenty patients with small cell carcinoma of the lung resistant to primary therapy, or relapsing after an objective response to initial treatment, were treated with vinblastine plus high-dose cisplatin chemotherapy. One patient achieved a partial remission lasting 4 months, while 19 patients had no objective response to this regimen. The median survival from the time of disease progression to death was 2.5 months (range, 1–7), with an overall median survival of 13 months (range, 5–31; diagnosis to death). In our patients, vinblastine plus cisplatin given as salvage therapy did not produce a significant response rate or survival prolongation. [Cancer Treat Rep 67:831–832, 1983]

Despite encouraging results with initial combination chemotherapy regimens, patients with resistant or relapsed small cell carcinoma of the lung (SCCL) have a short survival (1). We designed this study to assess the efficacy of vinblastine plus high-dose cisplatin as a salvage regimen in previously treated patients with progressive SCCL.

MATERIALS AND METHODS

Between March 1980 and September 1981, 20 patients with resistant or histologically documented relapsed SCCL were evaluated for this study at the University of Chicago or Michael Reese Hospitals. All patients initially presented with limited (stage III-M0) or extensive (stage III-M1) SCCL (1); they were treated with at least two cycles of combination chemotherapy consisting of 1000 mg/m^2 of cyclophosphamide (C) iv on Day 1, 40 mg/m^2 of doxorubicin (A) iv on Day 1, 30 mg/m^2 of methotrexate (M) iv on Day 22, and 100 mg/m^2 of etoposide (V) on Days 1 and 8 or 2 mg/m^2 of vincristine (O) iv on Day 1 (V-CAM or MOCA). Eight of nine patients presenting with limited disease received two cycles of V-CAM, followed by 4000 rads to the primary tumor site and mediastinum, and 2000 rads of prophylactic radiation therapy to the whole brain, after which chemotherapy was given monthly until disease progression.

Patients presenting with extensive disease received V-CAM or MOCA chemotherapy monthly until progression. Patients resistant to initial chemotherapy or presenting at the time of their first relapse after an initial response were eligible for this study. At the time of progression, all patients had tumor lesions measurable by physical examination, x-ray, or nuclide scan. Chemotherapy consisted of 0.2 mg/kg of vinblastine sulfate given by iv injection on Day 1, and 100 mg/m^2 of cisplatin given by iv infusion over 4 hours on Day 2. Cisplatin was administered with 24 hours of vigorous iv hydration and mannitol diuresis. Cycles were repeated every 21 days. Therapy was delayed for a wbc count < 3500/mm^3 or a platelet count < 75,000/mm^3 until counts exceeded these levels. Chemotherapy was discontinued after two cycles if further progression of SCCL was obvious on the basis of the entry parameters. Four patients presenting with evidence of brain metastasis received concurrent radiation therapy to the whole brain (4000 rads). However, those patients had measurable progressive disease in at least one other site outside the CNS. All patients were followed until death.

RESULTS

Characteristics of the patients are shown in table 1.

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Response and Survival

The response to initial treatment is shown in table 1. Responders (complete and partial) received an average of seven cycles of V-CAM or MOCA (range, 2-13), while nonresponders received three cycles of initial chemotherapy at most. The median duration of initial response (17 patients) was 8 months. Seven patients had disease progression confined to the chest (primary tumor site or mediastinum), while 13 had progression in one or more distant metastatic sites (liver, six patients; brain, four; bone, three; and lymph nodes, abdominal mass, and skin, one each).

One patient with limited disease and no initial response to V-CAM chemotherapy, and who received no radiation therapy, had a partial response (> 50% regression of all measurable disease) to vinblastine plus cisplatin therapy lasting 4 months, after which osseous metastases developed. Nineteen patients had no objective response to this salvage regimen. Patients received an average of two cycles of vinblastine and cisplatin (range, 1-4). The median survival from the time of progression and the initiation of vinblastine and cisplatin therapy was 2.5 months (range, 1-7). The overall median survival of the entire group was 13 months (range, 5-31, initial treatment to death). All patients died with evidence of progressive SCCL.

TABLE 1.—Characteristics of patients receiving vinblastine + cisplatin

Characteristic	No. of patients
Sex	
Male	12
Female	8
Median age in yrs (range): 58 (40-73)	
Median Karnofsky performance status (range): 70 (60-80)	
Initial stage	
Limited (III-M0)*	9
Extensive (III-M1)	11
Initial therapy	
V-CAM	14
MOCA	6
Response to initial therapy	
Complete response	8
Partial response	9
No response	3
Median duration of initial responses in mos (range): 8 (4-16)	
Sites of progressive disease	
Local	7
Metastatic	13

*8 of 9 patients with limited disease received radiation therapy as part of their initial treatment.

Toxicity

All patients experienced some degree of nausea and vomiting with cisplatin administration. The median nadir wbc and platelet counts were 2000 and 85,000/mm^3, respectively. There were two episodes of severe neutropenia (wbc count < 500 cells/mm^3), and one patient died of sepsis while neutropenic (with evidence of progressive SCCL). No patients experienced a significant increase in BUN or serum creatinine, or a decrease in creatinine clearance.

DISCUSSION

The concept of non-cross-resistant chemotherapy regimens has been applied in the treatment of SCCL, both as initial and salvage therapy (2,3). In the past, cisplatin and vinblastine have been reported to have some single-agent activity in SCCL (4,5). We employed vinblastine plus cisplatin as a potentially non-cross-resistant salvage regimen. Despite the relatively good performance status of our patients and the fact that seven of the 20 patients had localized disease progression, the vinblastine plus cisplatin regimen provided only one partial response which was of short duration. With this one exception, response was uniformly poor and there were no prognostic factors favoring response or even stabilization of disease. Furthermore, myelosuppression was moderate to severe, perhaps owing to the previous treatment. In our experience, and that of other investigators, SCCL is usually responsive to initial chemotherapy. Patients who do not achieve remission or who relapse after an initial response, however, have a short survival (1,6). Based on our experience, vinblastine plus cisplatin is not effective in inducing remission or prolonging overall survival in previously treated patients with progressive SCCL.

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Local Administration of Interferon for Malignant Brain Tumors[1]

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We investigated the effects of local administration of interferon (IFN) on 13 patients with recurrent brain tumors. Histologic diagnoses were glioblastoma (eight patients), medulloblastoma (one), ependymoma (one), ependymoblastoma (one), pontine glioma (one), and astrocytoma (one). When tumor recurrence was evident local administration of IFN was started through an Ommaya reservoir, which was placed during repeat craniotomy. No tumor regressions were seen in the patients given weekly injections of IFN; however, in two of six patients given daily injections, a decrease of tumor volume and augmentation of natural killer activity were seen. [Cancer Treat Rep 67:833–835, 1983]

Since interferon (IFN) has been recognized as an antitumor agent in addition to an antiviral agent, clinical trials of IFN therapy for various malignant tumors have been increasing. We have already reported the results of IFN therapy by general administration for patients with malignant brain tumors (1). However, it was found that general administration is not always effective. On the other hand, local administration of IFN for various kinds of tumors, ie, malignant melanoma (2) and neuroblastoma (3), appears to be effective when given into or near the tumor tissue. In this paper, we report the results of IFN therapy, by local administration through intrathecal injection or into the dead space resulting from tumor removal, for patients with malignant brain tumors.

MATERIALS AND METHODS

In this study, we used human interferon-α (4), which was produced by the Department of Microbiology, Kyoto Prefectural University of Medicine. The specific activity of IFN was $> 1.1 \times 10^7$ IU/mg of protein (standard, World Health Organization No. 69/19).

Thirteen patients (ten adults and three children) were selected at random for this study (table 1). Craniotomy was performed in all patients except for the one with pontine glioma. All patients had completed ^{60}CO irradiation therapy. Basically, IFN therapy was started when any sign of tumor recurrence was manifested by clinical symptoms and/or CT scans; in some cases, craniotomy was again performed. After the tumor was excised, an Ommaya reservoir was placed over the skull surface, and the tube was inserted into the dead space resulting from tumor removal.

IFN (10^6 IU) was administered through the Ommaya reservoir or by intrathecal injection (table 1). In the first group (seven patients), IFN was administered once or twice/week (weekly group). In the second group (six patients), IFN was administered every day for 1 month followed by a 1-month suspension of administration (daily group). Local administration of IFN was combined with general administration in three cases and with chemotherapy in six cases. The average total dose of IFN administered was 12×10^6 IU in the weekly group and 25×10^6 IU in the daily group. IFN in the serum and cerebrospinal fluid at 3, 6, 12, and 24 hours after local administration and in the aspirated fluid from the tumor bed at 3, 6, and 24 hours after local administration was examined by a dye uptake method (5) using FL cells and vesicular stomatitis virus. Though no IFN was detected in the serum or cerebrospinal fluid, aspirated fluid revealed $2.6–9.6 \times 10^4$ IU/ml of IFN even 24 hours after the local administration.

Natural killer (NK) activity measured by ^{51}Cr releasing assay (6) using K562 target cells (E/T ratio, 10:1) was checked periodically. Serial computerized tomographic (CT) scans were performed, and the growth

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TABLE 1.—Local administration of IFN

Patient No.	Age (yrs)/ sex	Tumor type	IFN-α administration			Combined chemotherapy	Systemic IFN administration ($\times 10^6$ IU)	Side effect	Response†
			Interval*	Total dose ($\times 10^6$ IU)	Ommaya reservoir				
1	5/F	Ependymoma	Weekly	7.25	+	—	—	Fever, meningitis	W
2	38/F	Glioblastoma	Weekly	16	–	Methotrexate	—	None	W
3	54/M	Glioblastoma	Weekly	29	–	Methotrexate	—	None	U
4	40/M	Glioblastoma	Weekly	6	+	Methotrexate	—	None	W
5	49/M	Glioblastoma	Weekly	14	+	Methotrexate	—	Fever	W
6	56/M	Glioblastoma	Weekly	4.5	+	—	1.2 im	Meningitis	W
7	12/F	Medulloblastoma	Weekly	8.9	–	Methotrexate	81 iv	Fever	W
8	57/M	Glioblastoma	Daily	25	+	—	—	Fever	PR
9	41/F	Glioblastoma	Daily	78	+	—	—	None	PR
10	7/M	Pontine glioma	Daily	29.6	–	ACNU‡/vincristine	306.6 iv	Fever, meningitis	U
11	48/M	Astrocytoma	Daily	24	+	—	—	None	U
12	8/M	Ependymoblastoma	Daily	25	+	—	—	None	W
13	50/M	Glioblastoma	Daily	25	+	—	—	None	W

* Weekly = 1×10^6 IU once or twice/wk; daily = 1×10^6 IU/day.

†PR = partial remission; U = unchanged; W = worsened. Therapeutic response was estimated in 3-6 mos after treatment.

‡Nimustine.

curve of the tumor was estimated from the summation of enhanced area in each CT film.

RESULTS

In general, there were no serious side effects. In five of 13 patients, transient fever > 38°C was observed for a few hours after IFN injection. Bacterial meningitis, occurring in three of 13 patients, caused the discontinuation of IFN therapy but was cured by the administration of antibiotics within a few weeks. Neurologic status and Karnofsky performance scale did not show any remarkable improvement.

In the daily group, tumor volumes of Patients 8 and 9 decreased to < 50% of the initial tumor volume between 4 and 5 weeks from the beginning of treatment. Tumor volumes of other patients in the daily and weekly groups did not show a decrease. Thus, partial remission estimated by CT scan was seen in only two of six patients in the daily group.

There were no remarkable changes of immunologic parameters. However, NK activities were augmented by local administration of IFN in the daily group. After IFN administration, NK activities of Patients 8, 9, 10, and 11 were twice as high as those before IFN therapy.

DISCUSSION

Both direct and indirect antitumor effects of IFN have been discussed. Recently, attention has been paid to the augmentation of NK activity as an indirect antitumor effect of IFN (6). Indeed, NK activity and other immunologic parameters were augmented by the general administration of IFN (1), but this method of administration was not always effective for malignant brain tumors with respect to the inhibition of tumor growth. Therefore, we investigated the local administration of IFN into the tumor bed as an alternative to general administration.

Immunologic parameters, except for NK activities, did not show any significant changes. However, NK activities were augmented by local administration of IFN only in the daily group. The values were as high as those achieved by general administration. Therefore, daily local administration of IFN might be sufficient for augmentation of NK activities.

Our results of local administration of IFN were almost the same as those previously reported for general administration (1). Although there was no complete remission of tumor by either local or general administration of IFN alone, we consider that IFN therapy will be a useful adjuvant therapy to inhibit tumor growth and augment NK activity.

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Phase II Trial of PCNU in Non-Small Cell Lung Cancer[1,2]

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PCNU is an iv administered lipid-soluble nitrosourea. Preclinical studies established its low carbamoylating and high alkylating activities, correlates of nitrosourea activity in murine models (1,2). In phase I trials myelosuppression was the dose-limiting toxic effect, with thrombocytopenia being more prominent than leukopenia (3–5). A phase II administration schedule of 100–125 mg/m^2 iv divided over 5 consecutive days, with repeat courses every 6 weeks, was recommended (3).

PATIENTS AND METHODS

Nineteen patients with measurable non-small cell lung cancer were entered in this phase II trial. Characteristics of the 17 evaluable patients are summarized in table 1. Eight patients had had no prior chemotherapy, and no patient had previously received a nitrosourea. PCNU was administered by slow iv infusion over 20 minutes at a dose schedule of 100–125 mg/m^2 (20–25 mg/m^2/day for 5 consecutive days) every 6 weeks. Standard response criteria were employed.

TABLE 1.—Characteristics of 17 patients evaluable for response

Characteristic	No. of patients
Median age in yrs (range): 56 (32–72)	
Median Karnofsky performance status (range): 70 (50–80)	
Men	11
Women	6
Type of disease	
Adenocarcinoma	14
Epidermoid carcinoma	3
Prior chemotherapy	9*

* None had had prior nitrosourea.

RESULTS

There were no major objective responses in 17 adequately treated patients (median No. of PCNU courses, one; range, one to three). Myelosuppression was the only significant drug-related toxic effect observed. The median wbc count nadir was 4400/mm^3 (range, 600–10,900), and the median platelet count nadir was 57,500/mm^3 (range, 12,000–390,000). These nadirs were generally seen 5 weeks after drug administration.

CONCLUSION

With no objective responses observed in this group of patients, the predicted true response rate is $< 16.8\%$ ($P = 0.05$). We conclude that PCNU, at the dose and schedule administered, does not have useful activity in this population of patients with non-small cell lung cancer.

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Phase II Study of Vindesine Infusion in Visceral Metastatic Malignant Melanoma[1]

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Recent reports have suggested that the synthetic vinca alkaloid vindesine may have significant activity in patients with malignant melanoma (1,2). Response rates may be as high as those of the most active agent dacarbazine, with much less toxicity. This paper describes the results of a phase II study of this drug.

PATIENTS AND METHODS

Patients with stage II and III progressive metastatic malignant melanoma were treated with dacarbazine and dactinomycin as first-line therapy at this institute. Patients who relapsed following this treatment or who failed to respond were entered in this study.

Vindesine was administered at a dose of 3 mg/m^2 as a continuous infusion in normal saline over 24 hours. A second group of patients received 6 mg/m^2. Infusions were repeated at weekly intervals. Therapy was delayed until recovery if the wbc count fell to $< 3.0 \times 10^9$/L or if the platelet count fell to $< 100 \times 10^9$/L. Complete and partial responses, stable disease, or disease progression were defined according to standard criteria of the International Union Against Cancer.

RESULTS

Sixty-three courses of vindesine were administered to 15 patients at 3 mg/m^2, and an additional three patients received seven courses at 6 mg/m^2. All of the patients had progressive visceral metastases (table 1). Toxicity was not a reason for discontinuing therapy in any patient. Karnofsky performance status was not improved by the treatment, and no responses were seen in any of the 15 patients. In every patient, the reason for discontinuing therapy was progressive disease. The probability that the true response rate is $\geq 20\%$ is therefore 0.95. Only one patient remains alive, and that patient has stopped therapy because of progressive disease. The median survival from the beginning of treatment was 2.5 months (range, 2–9).

TABLE 1.—Patient characteristics and sites of metastasis*

Age in yrs	
Median	53
Range	23–73
Performance status	
Median	60
Range	50–70
No. of courses given	
3 mg/m^2	
Median	3
Range	3–9
6 mg/m^2	
Median	2
Range	2–3
Site of metastases	
Skin	10
Nodes	15
Liver	6
Lung	9
Bone	5
Soft tissue	9

* Unless specified otherwise, values = No. of patients.

DISCUSSION

An analysis of the reported phase II studies of vindesine in malignant melanoma shows that many of the responses have occurred in nonvisceral sites (1,2). A response in these sites does not significantly affect the development of visceral metastases or overall survival. We wanted to determine the response rate in patients with visceral malignant melanoma but who still had a performance status of ≥ 50.

It seems logical to administer vindesine over a prolonged period since it is a phase-specific agent. More cells will enter the susceptible phase of the cell cycle, and cell

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kill will be maximized. A continuous infusion of 3 mg/m^2 of vindesine is known to give serum levels similar to those given by a bolus injection, without the high initial peak levels (3). Before proceeding to longer infusions we wanted to establish the efficacy of a 24-hour infusion, in terms of both response rate and tolerability (with particular regard to phlebitis).

Our results are clearly most disappointing. The true response rate must be < 20%. Vindesine is probably less effective than dacarbazine in patients with visceral metastases. We would therefore counsel caution in using vindesine as first-line therapy for patients with visceral metastases from malignant melanoma.

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Phase II Trial of Bisantrene for Unresectable Non-Small Cell Bronchogenic Carcinoma: An Illinois Cancer Council Study[1,2]

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Bisantrene is a guanidino-substituted anthracene derivative with deletion of the glucosamine side chain. It entered phase I studies utilizing two schedules: a single dose every 3–4 weeks (1,2) and a daily × 5 dose every 4 weeks (3). The recommended schedule for phase II evaluation was a single infusion over 2 hours every 3–4 weeks starting at a dose of 260 mg/m^2. Although no activity for bisantrene in non-small cell bronchogenic carcinoma was observed in phase I studies, a phase II evaluation was recommended since non-small cell bronchogenic carcinoma represents one of the signal tumors in the clinical panel of the Division of Cancer Treatment, National Cancer Institute.

Thirty patients who had advanced non-small cell bronchogenic carcinoma with one or more previously nonirradiated lesions measurable in two perpendicular dimensions were entered into this study. Bisantrene (260 mg/m^2) was dissolved in 500 ml of 5% dextrose in water and infused over 2 hours through a peripheral vein. Standard criteria were used to assess response, with the following additions: (*a*) loss of ≥ 10% of pre-entry body weight constituted disease progression, (*b*) decrease of pre-entry performance status by two or more levels constituted progression, and (*c*) 50% decrease in a 5-cm filling defect in the liver scintigram or 30% decrease in the sum of liver measurements taken below the xiphoid process and the subcostal margin at the midclavicular line constituted partial response. An adequate trial for response was defined as one dose, with survival for at least 14 days (three patients died before 14 days). Evaluation for toxic effects was performed on all treated patients (one patient refused treatment).

Patient characteristics, responses, and toxic effects are detailed in table 1. No responses were seen in 26 evaluable patients. The relative lack of myelosuppression in this study is probably due to the rapid progression of tumor, such that fewer than one-half of the patients received more than one course of therapy with a planned dose escalation if toxicity was not observed with the initial course. Phlebitis was not a major problem, although it was still observed even with increased drug dilution and delivery time.

Two patients developed a syndrome of fever and tachycardia without hypotension 24–36 hours following bisantrene infusion. One of these patients had recurrence of the syndrome with the second course of therapy.

We conclude that bisantrene does not have activity (≤ 15% response rate with 95% confidence, probability < 0.015) in unresectable non-small cell bronchogenic carcinoma.

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TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)*
Age in yrs	
Median: 58.5	
Range: 44–77	
Performance status	
Median: 1	
Range: 0–2	
Sex	
Male	23 (77)
Female	7 (23)
Cell type	
Adenocarcinoma	12 (40)
Squamous cell	10 (33)
Large cell	8 (27)
Prior therapy	
None	22 (73)
Radiation	6 (20)
Chemotherapy	1 (3)
Combined	1 (3)
Indicator lesion	
Lung	19 (63)
Node	5 (17)
Liver	4 (13)
Abdominal mass	1 (3)
Multiple	1 (3)
Courses of therapy	
None	1 (3)
1	15 (50)
2	10 (33)
3	4 (13)
Progression documentation†	
25% increase in indicator	10 (38)
New lesion	12 (46)
Weight loss of 10%	2 (8)
Decreased performance status (2 levels)	2 (8)
Response†	
Partial or complete	0
Stable disease	0
Disease progression	26 (100)
Toxic effect‡	
Drug death	0
Leukopenia (wbc count < 4000/μl)	8 (28)
Thrombocytopenia	0
Nausea or vomiting	10 (34)
Phlebitis	4 (14)
Fever and tachycardia	2 (7)
Hypotension	4 (14)
Subsequent therapy	
None	25 (83)
Chemotherapy	5 (17)
Partial response	2 (40)

*Unless specified otherwise, N = 30.
†N = 26.
‡N = 29.

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Phase II Trial of Acivicin in Advanced Metastatic Breast Cancer[1]

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The antimetabolite antibiotic acivicin, a glutamine antagonist, has demonstrated significant antitumor activity in a variety of animal models, including the MX1 human mammary tumor xenograft system (1-3). Phase I evaluation of an iv 5-day schedule defined a maximal dose of 15-18 mg/m²/day repeated every 3 weeks to be tolerable for a phase II trial (4). Myelosuppression and CNS toxic effects appeared dose-limiting. Because of present limitations with current second-line treatment programs in metastatic breast cancer, we elected to evaluate acivicin in patients with refractory metastatic breast cancer who have failed standard therapy.

MATERIALS AND METHODS

Eligibility criteria for this study included a performance score of 3 or better (International Union Against Cancer criteria), presence of measurable lesions, and informed consent. Patients with known CNS disease were not excluded. Based on our phase I study, a starting dose of 15 mg/m² administered iv over 30 minutes daily for 5 days every 3 weeks was given to all patients with adequate bone marrow reserve and normal liver function (bilirubin < 2 mg/100 ml). Poor-risk patients (inadequate bone marrow reserve, known CNS disease) were given a starting dose of 12 mg/m²/day. Patients with compromised liver function (ie, bilirubin > 2 mg/100 ml) were started at 9 mg/m²/day.

RESULTS

Twenty-four patients were entered in the study. Two patients were not evaluable: one patient died within 2 weeks from the initiation of therapy with progressive disease, and another patient, although entered in the study, never received therapy with acivicin. The characteristics of the 22 evaluable patients and the toxic effects observed are outlined in table 1. Neurologic and gastrointestinal toxic effects were dose-limiting in six patients (27%). Acute, readily reversible bronchospasm was seen in one patient immediately following completion of the second dose of her second course of therapy, resulting in discontinuation of further treatment with acivicin. No other life-threatening or serious toxic effects were encountered. Most patients received a starting dose of 12 or

TABLE 1.—Patient characteristics and toxic effects

No. of evaluable patients	22
Median age in yrs (range)	47 (30-63)
Median Zubrod performance status (range)	2 (0-3)
Prior chemotherapy	
Median No. of regimens (range)	4 (1-9)
Median duration in mos (range)	20 (6-51)
Median No. of metastatic sites (range)	3 (1-4)
Dominant site	
Soft tissue	1
Visceral	22
Toxic effects	
Hematologic (× 10^3 cells/mm³)	
Median wbc count nadir (range)	3.5 (0.9-9.7)
Median granulocyte count nadir (range)	2.2 (0.4-6.3)
Median platelet count nadir (range)	198 (4-487)
Neurologic*	
Lethargy	8 (36)
Confusion	7 (32)
Sleepiness	3 (13)
Light-headedness/dizziness	2 (9)
Memory difficulty	2 (9)
Psychotic state	1 (4)
Depression	1 (4)
Difficulty with speech	1 (4)
Restlessness	1 (4)
Gastrointestinal*	
Nausea and vomiting	6 (27)
Diarrhea	3 (13)
Mucositis	3 (13)
Generalized muscular aches*	1 (4)
Acute bronchospasm*	1 (4)
Fever of unknown origin/infection*	1/1 (4/4)

*Values = No. of patients (%).

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15 mg/m^2/day for 5 days, and doses were escalated to 18 mg/m^2 in five courses. There were no complete or partial responses, five patients had stable disease, and 17 patients had progressive disease (95% confidence interval, 0–13).

DISCUSSION

This phase II trial failed to identify any activity of acivicin in heavily pretreated patients with metastatic breast cancer. The degree of myelosuppression experienced suggests that the doses used in this study were somewhat low and that further dose escalation may be feasible. Nevertheless, on the basis of our data, acivicin appears to have no activity given in the dose range of 9–18 mg/m^2 on a daily × 5 schedule.

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Phase II Study of Amsacrine (AMSA) in Previously Treated Patients With Advanced Transitional Cell Carcinoma of the Urothelium: A National Bladder Cancer Collaborative Group A Study[1,2]

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For the National Bladder Cancer Collaborative Group A

The substituted acridine derivative amsacrine (AMSA) exerts antiproliferative activity by intercalation and external binding to DNA (1). Phase I studies indicate that the primary toxic effect of AMSA is myelosuppression when a dose of 90–120 mg/m^2 is administered. Antitumor activity has been reported in acute leukemia, esophageal and ovarian cancers, and Hodgkin's disease (2–5). This report describes the results of a phase II trial of AMSA conducted by the National Bladder Cancer Collaborative Group A in patients with metastatic transitional cell carcinoma of the urothelium.

METHODS

Fifteen patients were treated and were evaluable for response and toxicity. Patients had either bidimensionally measurable disease (11 patients) or disease which was clinically evaluable on cystoscopy and computer-assisted tomographic scan (four), and all had histologically proven, advanced transitional cell carcinoma (bladder primary, 14 patients; renal pelvis primary, one). All patients had previously failed at least one systemic chemotherapy regimen, which included cisplatin. Hematologic, hepatic, and renal function parameters were normal before therapy and patients had an Eastern Cooperative Oncology Group performance status of 0, 1, 2, or 3. AMSA was administered iv at a dose of 90 mg/m^2, weekly hematologic parameters were determined, and therapy was repeated every 3 weeks. The dose of AMSA was increased to 120 mg/m^2 if no significant myelosuppression was noted after the first dose. Seven of the 15 patients received one or more doses of AMSA at the 120-mg/m^2 level. Fourteen patients received two or more doses of AMSA.

RESULTS

Toxicity (table 1)

Mild myelosuppression was the only clinically important toxic effect of AMSA. No patient experienced a wbc count < 2000/μl; one patient developed thrombocytopenia (36,000 cells/μl on Day 10), which rapidly recovered. Four patients had mild or moderate nausea and vomiting. Transient elevation of the serum bilirubin to 2.9 mg/dl occurred following the first dose of AMSA in one patient. This rapidly corrected and did not recur following two subsequent 90-mg/m^2 doses of AMSA. No cardiac or renal toxicity was noted.

Response

No patient experienced significant tumor reduction following administration of AMSA. All 15 patients developed progressive disease between 3 and 17 weeks (mean, 9) following the initiation of AMSA. With none of 15 previously treated patients responding to treatment, the true response rate of AMSA in transitional cell carcinoma is < 20%, with 95% certainty.

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DISCUSSION

This phase II study shows that AMSA administered at this dose and schedule has little or no activity in previously treated patients with advanced transitional cell carcinoma. Further studies of AMSA at doses of 90–120 mg/m^2 in patients with transitional cell carcinoma of the urinary tract previously treated with systemic chemotherapy are not indicated.

TABLE 1.—Evaluation of AMSA (90 mg/m^2) in transitional cell carcinoma

	No. of patients
Total	15
Prior systemic cisplatin treatment	15
Prior pelvic radiation	8
Wbc count nadir < 2000/μl	0
Platelet count nadir < 100,000/μl	1*
Nausea (mild-moderate)	4
Objective responses	0
Time to treatment failure	
Range, 3–17 wks	
Mean, 9 wks	

*36,000/μl.

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Etretinate, Lomustine, and Bleomycin Versus Lomustine and Bleomycin in Advanced Squamous Cell Lung Carcinoma[1]

Retinoids (natural vitamin A substances and synthetic analogs) form a new class of substances which are active prophylactically and therapeutically in epithelial tumors of animal and man (1).

Etretinate (Tigason) is the first available aromatic retinoic acid derivative. Clinical trials with this substance in squamous cell carcinomas of different organs have been unsatisfactory so far, because active dose levels (2–3 mg/kg/day) led to unacceptable toxicity of skin and mucous membranes (1). Therefore, in this trial, it was our goal to combine etretinate at an acceptable dose (1 mg/kg/day) with two active single drugs, lomustine and bleomycin, in squamous cell lung cancer (SCLC).

MATERIALS AND METHODS

Patients with histologically proven SCLC whose disease was no longer amenable to curative surgery and who had at least one measurable lesion were eligible, after giving informed consent. Histologic material was reviewed by K. M. Müller.

Patients with the following conditions were not eligible for study: (*a*) age > 75 years, (*b*) Karnofsky index < 40, (*c*) prior chemotherapy with any of the agents to be used in the study, (*d*) 4 weeks from latest radiotherapy or chemotherapy, (*e*) circumstances not permitting at least 6 weeks of treatment and follow-up, (*f*) leukocyte count < 4000/mm³, (*g*) platelet count < 100,000/mm³, (*h*) septicemia or severe infection, (*i*) renal impairment (BUN > 20 mg/100 ml or creatinine >1.4 mg/100 ml), (*k*) clinically significant impairment of hepatic function (bilirubin > 2 mg/100 ml or transaminases > three times above the upper normal value), and (*l*) any neurologic abnormality.

Eligible patients were randomly assigned to receive Regimen 1 (ECB): etretinate (1 mg/kg/day orally), lomustine (120 mg/m² orally every 6 weeks), and bleomycin (15 mg/m² by iv push twice weekly for 6 weeks, then 15 mg/m² once weekly up to a maximal total dose of 450 mg); or Regimen 2 (CB): lomustine and bleomycin at the dose and application as in Regimen 1. Evaluation of response parameters was made every 3 weeks. Responders and patients with stable disease continued to receive the same therapy, while nonresponders were removed from the study after 6 weeks.

Response rates were compared using Fisher's exact test and chi-square analysis (2). Survival curves were analyzed by the BMDP-77 Series programs 1 L (3).

Leukocyte toxicity was rated as follows: 1+ (counts between 3000 and 4500/mm³), 2+ (counts between 2000 and 2999/mm³), and 3+ (counts between 1000 and 1999/mm³). Platelet toxicity was rated as follows: 1+ (counts between 90,000 and 130,000/mm³), 2+ (counts between 50,000 and 89,999/mm³), and 3+ (counts between 25,000 and 49,999/mm³). Nausea and vomiting was rated 1+ if it did not require treatment, 2+ if it could be controlled by antiemetics, and 3+ if it could not be controlled. Toxicity of skin and mucous membranes was rated subjectively as 1+ (mild), 2+ (moderate), and 3+ (severe). Chi-square testing with Yates correction in fourfold tables was applied.

RESULTS

Fifty-eight patients (51 males and seven females) were entered in the study. Twenty-seven patients (age: < 40 years [two patients]; 40–59 years [nine]; and 60–75 years [16]) received ECB. Thirty-one patients (age: < 40 years [one patient]; 40–59 years [eight]; and 60–75 years [22]) received CB. Twelve patients (six from each group) were inevaluable because of early death due to progressive cancer because they had lung cancer of nonsquamous cell histology and because of loss to follow-up (2). In 46 evaluable patients, the Karnofsky index was 90–100 in two patients, 70–80 in 21, 50–60 in 21, and 40 in two.

Response

There were no complete or partial responses. Minor responses (reduction between 25% and 49% in the sum of the products of perpendicular diameters in all lesions) occurred in five patients (24%) receiving ECB and in three patients (12%) receiving CB. Minor responses lasted from 21 to 108 days (median, 35), and

[1]Conducted by the Cooperative Group for Medical Oncology in the German Cancer Society (AIO). The following members participated in the study: W. Weber, Gh. Germano, and J. P. Obrecht (Basel, Switzerland); H. Arnold (Freiburg, W Germany); P. Drings (Heidelberg, W Germany); W. Queisser (Mannheim, W Germany); K. M. Müller (Münster, W Germany); and M. Westerhausen (Duisburg, W Germany).

TABLE 1.—Toxic effects among 21 patients given ECB and 25 patients given CB

Toxic effect	No. of patients with side effects after receiving—		*P* value
	ECB	CB	
Skin erythema			
1+	4	3	
2+	3	1	0.021
3+	6	2	
Skin scaling			
1+	8	5	
2+	1	1	0.021
3+	4	0	
Mucous membrane dryness			
1+	5	9	
2+	6	1	0.132
3+	3	0	
Nausea			
1+	7	11	
2+	1	4	0.864
3+	4	1	
Vomiting			
1+	5	3	
2+	2	2	0.476
3+	1	1	
Leukopenia			
1+	3	5	
2+	3	3	0.826
3+	1	0	
Thrombocytopenia			
1+	7	7	
2+	3	2	0.825
3+	0	1	

did not correlate with degree of histologic differentiation. Stable disease (reduction or increase of < 25% without the appearance of new lesions) was observed in nine patients (43%) receiving ECB and in 13 patients (52%) receiving CB. There was no statistical difference between both groups. Median survival was 170 days in the ECB group and 146 days in the CB group. This difference is statistically nonsignificant.

Toxicity

Side effects of skin and mucous membranes (erythema, scaling, and dryness) occurred more frequently and were more severe in the ECB group (table 1). In addition, moderate stomatitis occurred in two patients receiving ECB and in one patient receiving CB, acral swelling occurred in two patients receiving CB, and nose bleeding occurred in one patient each. Nausea and vomiting was similar in both groups (table 1). Occasionally obstipation (two patients receiving ECB and one receiving CB), headaches (two patients receiving ECB and four receiving CB), and paresthesias (one patient in each group) were observed. Hematotoxicity was rare and mild (table 1). All of the toxic effects were reversible.

DISCUSSION

Our results with the ECB and CB regimens were disappointing, especially since all of the study drugs in this trial were thought to be active in SCLC. Interestingly, the course of the disease was stable to regressive in > 60% of all patients (67% of the ECB group and 64% of the CB group), independent of histologic grade. Dosage might have been suboptimal as indicated by the mild hematotoxicity.

Toxicity affected mainly skin and mucous membranes, particularly in the etretinate-containing regimen, and was always reversible. The treatment arms were not double-blinded, and observer bias can therefore not be excluded completely.

Despite these unsatisfactory results, retinoids deserve further testing because they are different in their mechanisms of action (stimulation of cell differentiation, inhibition, and reversal of cell transformation) from cytostatic agents.

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[2]Dr. W. Berchtold did the statistical tests in table 1.
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Irreversible Thrombocytopenia Following Aminoglutethimide

Aminoglutethimide is well-established as an effective drug in the management of advanced breast cancer, particularly in patients with bone metastases (1-3). Marrow depression is an infrequent side effect and is reversible on withdrawal of the drug (4,5).

We report a case of fatal thrombocytopenia following aminogluthethimide therapy. Although this was probably an idiosyncratic drug reaction, it may be wise to monitor marrow performance during drug induction.

CASE REPORT

In 1978, a 47-year-old premenopausal woman presented with a T4 N2 M0 medullary carcinoma of the breast. Primary treatment consisted of radiotherapy sandwiched between courses of combination chemotherapy (cyclophosphamide, methotrexate, 5-FU, doxorubicin, and vincristine). This produced local and systemic control for 2 years. After this time, disease recurred in the breast itself and spread to the contralateral breast and axilla; in addition, the patient complained of back pain. After an unsuccessful trial of tamoxifen, it was decided to try aminogluthethimide. Pretreatment investigations showed normal chest, lumbar spine, and pelvic radiographs; normal bone scan; slightly raised alkaline phosphatase of 240 IU/L; hemoglobin of 11.3 g/100 ml; platelet count of 235,000/mm^3; and wbc count of 6200/mm^3 with a normal differential and film. Therapy was started with 250 mg of aminoglutethimide together with 10 mg of prednisolone three times a day. Two weeks later the patient was admitted to the hospital with increasing bone pain, nausea, hematuria, bleeding from her ulcerated breast lesion, and petechial skin hemorrhages. Her blood cell count now showed the following profile: hemoglobin of 8.5 g/100 ml, platelet count of 38,000/mm^3, and wbc count of 5200/mm^3 with normal clotting times. The aminoglutethimide was stopped and the patient was given fresh whole blood and platelet transfusions. Unfortunately, she continued to bleed despite repeated transfusions and died the following week. The platelet count never rose > 40,000/mm^3 (nadir, 18,000). Permission for marrow aspiration and necropsy was not granted.

The major side effects of aminoglutethimide include lethargy in 37% of patients, rash in 26%, ataxia in 12%, and nausea in 5% (1-3). These usually occur during the induction period and can be reduced by starting the drug at reduced dosage (1). Marrow toxicity occurs in 4%-12% of patients and is usually mild (1,3). Cases of pancytopenia and thrombocytopenia have been reported, but each responded to withdrawal of the drug (4,5).

The case presented here is the first irreversible blood dyscrasia. Although marrow failure due to tumor invasion cannot be ruled out, it is likely that this represents idiosyncratic aminoglutethimide-induced marrow failure.

Since aminoglutethimide is recommended in patients with bony secondaries, it may be worthwhile to monitor marrow performance during induction. In our case this may have allowed us to stop the drug before the fall in the platelet count became symptomatic or life-threatening.

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Midwest Regional Oncology Conference

Kansas City, Missouri, October 20–21, 1983

The theme of this conference, to be held at the Alameda Plaza Hotel, is "Knowledge Today for Tomorrow's Challenge," and is sponsored by St. Luke's Hospital of Kansas City and American Cancer Society, Missouri Division, Inc. The audience will consist of RN's and other allied health professionals who care for cancer patients. The conference will meet the continuing educational needs of today's oncology nurse in preparation for tomorrow's challenge. The three keynote addresses will be presented by Connie Henke Yarbro, Judi Johnson, and Diane Fink.

The conference meets the criteria for 13 contact hours approved by the Missouri Nurses' Association. The fee for the two-day conference is $80 ($45 for one day).

For further information, contact: Jane Popp, Conference Manager, Center for Continuing Education, St. Luke's Hospital, Wornall Rd at 44th, Kansas City, MO 64111 (telephone 816-932-2220).

Second European Conference on Clinical Oncology and Cancer Nursing

Amsterdam, the Netherlands, November 2–5, 1983

This conference, hosted by the Koningin Wilhelmina Fonds, Organisation for Cancer Control, the Netherlands, will be the first gathering of all European Scientific Oncology Societies at one time and will be held in the International Congrescentrum RAI.

The scientific program will consist of four parallel sessions on different subjects in the morning and afternoon where selected proffered papers will be communicated and discussed. Plenary sessions will follow and the parallel sessions and posters relevant to the particular subjects will be reviewed.

There will be opportunity at this integrated conference for nurses and doctors to meet and discuss problems in common. Future developments in oncology nursing and possible collaboration of European nurses will be discussed.

A social program for participants and a special excrusion program for accompanying persons have been composed.

For further information, contact: Second European Conference on Clinical Oncology, c/o Organisatie Bureau Amsterdam bv, Europaplein, 1078 GZ Amsterdam, the Netherlands.

Current Concepts in Cancer Therapy, II

St. Louis, Missouri, November 3–5, 1983

This meeting will be presented by the Department of Surgery and the Office of Continuing Medical Education, Washington University School of Medicine in St. Louis. The program chairman is Marc K. Wallack, MD. The meeting will be accredited for 17 hours AMA Category 1, AAFP Prescribed, and AOA.

For further information, contact: Loretta Giacoletto, Office of CME, Washington University School of Medicine, Box 8063, 660 S Euclid, St. Louis, MO 63110 (telephone 314-454-3873).

New Perspectives in Human Lymphoma

Houston, Texas, November 9–12, 1983

This 27th annual clinical conference, held at the Shamrock Hilton Hotel, will focus on various controversial aspects of the diagnosis and management of lymphomas. Topics to be covered are pathogenesis and etiology, staging, and the use of various modalities of therapy, including, radiation, chemotherapy, immunotherapy, and bone marrow transplantation.

Speakers include: C. Berard, D. Bergsagel, C. Bloomfield, G. Bonadonna, R. Fisher, E. Frei, N. Gutensohn, M. Kadin, A. Levine, I. Magrath, L. Nadler, and L. Prosnitz. Conference co-chairpersons

are Drs. Richard Ford, Lillian Fuller, and Frederick B. Hagemeister.

For more information, contact: Office of Conference Services, Box 18, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030 (telephone: 713-792-2222).

International Symposium on Gynecologic Oncology, Surgery, and Urology

Miami, Florida, November 14–16, 1983

This symposium, designed to bring together gynecologists who are in surgical gynecologic practice with primary interest and experience in major gynecologic cancers, is sponsored by the University of Miami School of Medicine, Department of Obstetrics and Gynecology. This is not a review course, but rather an attempt to bring together specialists from different countries with divergent backgrounds to stimulate lively discussions on each topic. The presentations by the University of Miami faculty are designed to provide recent data and current clinical protocols to open discussion on controversies in selected aspects of their specialty. The program will be presented in English, German, and Spanish.

This course meets the criteria for 18 credit hours in Category I of the Physician's Recognition Award of the American Medical Association, and for 18 cognates, Formal Learning, by the American College of Obstetrics and Gynecologists. Tuition is $225.

For more information, contact: University of Miami School of Medicine, Department of Obstetrics and Gynecology R-116, PO Box 016960, Miami, FL 33101 (telephone: 305-547-6944 or OB-GYN Seminars 305-251-0591).

Roswell Park Memorial Institute Continuing Education Programs

Buffalo, New York, October–December, 1983

Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners to disseminate information on the newest methods in the diagnosis and treatment of cancer. Each monthly seminar is organized by a different department of the Institute covering a different clinical specialty. These seminars qualify for Category I credit toward the AMA Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $45.00 registration fee for 1-day seminars and a variable fee for longer programs. The seminars are cosponsored by the Cancer Control Program of the Institute and the American Cancer Society, New York State Division and Erie County Unit.

The seminars are scheduled as follows: Metastasis, *October 5–6, 1983* (Chairperson: Dr. Leonard Weiss); Molecular Events in Differentiation and Neoplasia, *November 10, 1983* (Chairperson: Dr. Timothy O'Connor); and Up-date in Neurological Oncology, *December 8, 1983* (Chairperson: Dr. Charles West).

For more information, contact: Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716-845-4406).

Association for Brain Tumor Research Applications for 1984 Fellowships

The Association for Brain Tumor Research (AFBTR), Chicago, is now accepting applications for fellowships from postdoctoral candidates whose intent is to pursue a career in brain tumor research. Preference will be given to MD's who are citizens or permanent residents of the US seeking research training that will enable them to pursue careers as clinical investigators.

AFBTR fellowships offer an award of $18,000 per annum plus a $2000 annual travel allowance for a period of two years, with a third year renewable option. Each year's travel must include one presentation to the AFBTR Board of Directors in Chicago.

All applications will be reviewed by the Association's Medical Advisory Council and awards will be made based on their recommendations. Criteria for selection will include the quality of the individual applicant; the quality of the program to which he/she is applying; and the research he/she is undertaking. Applications must be submitted by the sponsoring program director no later than *January 6, 1984*. Awards will be announced by April 1, 1984, for fellowships to begin July 1, 1984.

Application forms may be obtained from and completed applications should be mailed to: The Association for Brain Tumor Research, Suite 200, 6232 N Pulaski Rd, Chicago, IL 60646.

For more information, contact: Donald Segal, Executive Director, Association for Brain Tumor Research, 312-984-1000.

Third Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 18–25, 1984

For information, contact: Dr. Stephen E. Jones, Section of Hematology and Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Intra-Arterial and Intracavitary Chemotherapy 1984

San Diego, California, February 24–25, 1984

This continuing medical education conference will take place at the Holiday Inn at the Embarcardero (San Diego, CA). The conference is approved for 14 hours of AMA/CMA credit as well as AAFP and the fee is $250.

For more information, contact: Office of Continuing Medical Education, UCSD School of Medicine, M-017, La Jolla, CA 92093.

Sixty-Sixth Annual Meeting of the American Radium Society

San Diego, California, March 18–22, 1984

The meeting will take place at the Hotel Del Coronado in San Diego.

For more information, contact: Mrs. Sally R. Polek, Office of the Secretariat, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

Second International Conference on Malignant Lymphoma: Current Status and Prospects

Lugano, Switzerland, June 13–16, 1984

This conference, to be held at the Palazzo dei Congressi, will deal with all important approaches in the diagnosis and treatment of Hodgkin's and non-Hodgkin's lymphoma in children and adults. The lectures will be given primarily by invited speakers and selected controversial issues will be discussed by panels of leading experts. The program will include free presentations and a poster session.

Abstracts for free communications and the poster session, prepared according to the standard rules of AACR/ASCO, should be submitted to the address below no later than *January 15, 1984* for this conference. Abstracts will be selected for presentation by the conference organizing committee and advisory board. Abstracts for the two workshops (cytogenetics and enzymology of neoplastic lymphoid cells) may also be submitted.

For more information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland.

Position Vacancy: Associate Director, Radiation Research Program

The position of *Associate Director of the Radiation Research Program* is now vacant. This position is located within the Division of Cancer Treatment, National Cancer Institute (NCI), National Institutes of Health, Department of Health and Human Services.

This is a civil service position in the Senior Executive Service (SES), with a salary range from $56,945 to $63,800 per year. Candidates may be eligible for Physicians Comparability Pay up to $10,000 per year. (Alternatively, candidates may be eligible for appointment in the Commissioned Corps of the US Public Health Service.) The individual selected, if not presently in SES, must serve a 1-year probationary period.

The Associate Director for the Radiation Research Program has overall responsibility for the planning, evaluation, and coordination of broad national and international radiation research programs designed to determine the effectiveness of radiation in cancer treatment, usefulness in diagnosis of cancer and other diseases, and biological effects of exposures to low doses of ionizing and non-ionizing radiation. The Association Director also administers a $60 million contract and grant program.

Applications (personal qualifications statement SF-171) are to be sent to the address below. *Applications must be postmarked by November 15, 1983.* A current curriculum vitae and bibliography

must accompany all applications. The National Institutes of Health is an Equal Opportunity Employer.

For further information, contact: Cynthia A. Kauff, PMS, National Cancer Institute, Bldg 31, Rm 3A23, Bethesda, MD 20205 (telephone: 301-496-6862).

Cancer Treatment Symposia: Proceedings of the United States-Japan Meeting on Drug Development and Cancer Treatment Research

Lmited copies of this first issue of *Cancer Treatment Symposia* (Volume 1, 1983) are available without charge from the Division of Cancer Treatment, National Cancer Institute.

The US-Japan Cooperative Cancer Research Program, sponsor of the US-Japan meeting on Drug Development and Cancer Treatment Research (Gettysburg, PA, Nov 1981), is an important program in a series of bilateral agreements developed by the National Cancer Institute with other countries to encourage and support intensive international collaboration in cancer research. The Proceedings of this meeting document the recent exchange of information between Japan and the United States on the current status of new anticancer drug development in the two countries.

For a copy of this issue of Cancer Treatment Symposia, contact: Anne McCarthy, Editor, Cancer Treatment Symposia, National Cancer Institute, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 229, National Institutes of Health, Bethesda, MD 20205, telephone 301-496-6975.

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